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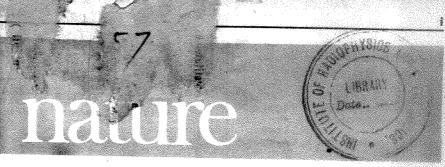
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The path to perfection. Saint Catherine's blemishes are layers of paint coming away from the wood, as detected by holographic interferometry.

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### Guide to authors

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### A hundred years ago



Some of the Paris newspapers announced that M. Wurtz, Dean of the Faculty of Medicine at Paris, would be obliged to resign; the Figuro went so far as to give the name of the intended successor of the celebrated Professor of Chemistry—a M. Depaul. The rumour happily has proved false, and was maliciously spread because a clerk employed in the office of the Faculty had been dismissed for misdemeanour. There is, however, to be a demonstration among the students in honour of M. Wurtz, who is a great favourite with them.

From Nature, 11, 5, November 5, 1874.

# nature

Volume 252

November 1, 1974

# Britain reviews its defence

THE results of the British defence review initiated after the February election will soon be published and now, with Labour safely returned, they are likely to be received favourably by the government. Already political vultures are surrounding the Ministry of Defence and pointing to what they could achieve if handed a tiny fraction of the money allotted for defence; the impressive list runs from better hospitals to more performances of operas. The snag is that it is most unlikely that defence spending will be trimmed by any significant amount and certainly not by the £1,000 million, or 30%, that the left wing has been demanding.

The early talk, and indeed that enshrined in Labour's recent election manifesto, was of bringing Britain's defence expenditure into line with that of its western European partners. Later in the election campaign, perhaps when the contents of the review had been made known to Labour politicians, the talk was more mutedly of not expanding expenditure and of carefully reconsidering overseas commitments when they come up for review. An uninformed guess, then, is that the review, far from revealing large areas of fat to be trimmed off, will simply say that Britain must be more modest in future. For the statistics on European comparisons show clearly that to talk of bringing expenditure into line with that of, say, France and Germany depends very much on what criterion one uses for being 'in line', and by one criterion, at least, Britain is irreproachably in step. The accompanying table provides some relevant figures on military spending in Western Europe.

Since 1950, military spending by European countries has grown relatively slowly, once inflation has been accounted for. Indeed in the years 1949-68 Britain's military expenditure grew on average by only 2.2% per year. France, with its nuclear development in the 1950s, showed a higher rate of 5% per year and West Germany,



re-arming, grew by 6% per year. But all three countries had, by the late 1960s, stabilised their expenditure, and defence, in real terms, was hardly growing at all. As the table shows, growth subsequently re-established itself; only France has managed to keep things in check. Not too much, however, should be made of this recent growth, which may well reflect the difficulty of preventing military costs from rising more rapidly than other costs in inflationary times rather than any deliberately expansive policy.

What is evident from the table is the serious weakness that a depressed gross national product (GNP) imposes on a country with global and nuclear pretensions. In other ways Britain's expenditure looks quite lean and there is no conscription, by contrast with France and Germany, not only to put up the expense of the forces but to pull young men out of productive (and taxpaying) occupations. It is the percentage-of-GNP indicator that makes Britain seem so far out of line; there are few other countries in the world which spend as much of their GNP on defence.

The question is thus not one of how to save a few million pounds here and there with small scale economies. It is much more fundamentally, should Britain go on spending defence money at the same per capita rate as her near neighbours, thereby providing highly sophisticated armaments for her forces, or should she recognise her economic limitations and aim to bring the figure of 4.9% down to nearer, say 3.5%?

Presumably major surgery, such as the elimination of Britain's nuclear capacity, is the only way that such a saving could be achieved in the next ten years, and this is not an option that is likely to have been considered seriously. This is a shame; the rationale for possessing nuclear weapons is not so strong that it could not profit by occasional exposure and criticism if only to ensure that a new generation, to whom CND is only an historical episode, is appraised of the disadvantages, as well as the supposed advantages of the possession of nuclear weapons. It would be unfortunate if these items of mass destruction came to be forgotten about by the public and thus subject to the pressures only of technological momentum and of those employed in their construction.

- Selection or commence and com	Population (million)	GNP (\$1000 million)			Military ex	penditure		Armed	forces	R & D
			Inflation 1969–73	\$ million	Growth 1969–73†	% of GNP	Per capita \$	(thousands)	% of all men aged 18-45	as of total expendi- ture
Britain	56	177	7.7	8,673	10.9	4.9	155	352	3.4	9.0
France	52	277	6.0	8,438	7.0	3.1	162	504	4.9	9.0
West Germany	62	385	5.2	11,291	9.9	2.9	182	475	4.0	5.1

† Not corrected for inflation

Sources: The Military Balance 1974-1975 (International Institute for Strategic Studies.); SIPRI Yearbook 1973.

Be warned that all comparisons of military expenditure suffer from variations in definitions. Thus this table should be seen as no more than general indication of comparisons. See page 102 of *The Military Balance* for more detail.

### Agriculture and sunspots

WORLD food reserves have, recently, been larger than the variability in production associated with the weather, and oscillations about the general upward trend of agricultural productivity could be ignored. It is now apparent, however, that urgent consideration has to be given to the impact of climatic changes on food production. The world's grain reserves have dwindled from the 1969 peak (19% of annual consumption) to only 7% of annual consumption, less than the variability of about 10% induced by the weather. Although many people have reported associations between the solar cycle and the weather, relatively little attention has been paid to the fact that the 11-year and the 22-year sunspot cycles could produce important modulations of agricultural productivity.

The world wheat production figures for 1949-73 can be used to show how the sunspot cycles appear to influence food production. Sunspot maxima occurred at the end of 1957 and again in 1968 and it seems significant that global wheat production in 1958 was greater than in each of the next five years, whereas that in 1968 was greater than the average of the next four years. In 1954 (a year of sunspot minimum) production was less than in any of the two preceding or the two following years. In North and Central America, for example, the crop in 1954 was smaller than any other year for which we have data; the average crop during the five previous years was 25% bigger than that of 1954.

The fact that wheat production in the Northern Hemisphere increases at around sunspot maximum can be deduced from the wheat figures for many countries. In the People's Republic of China, the annual production during 1956-58 (centred on the 1957 sunspot maximum) was 22% greater than during the period 1960-65; the total produced in 1958 has not been equalled in any year up to and including 1973. The average production in the Soviet Union during 1956-58 was, as in China, greater than during 1960-65; in 1958 the crop was 54% bigger than in 1963. The average wheat production in Canada during 1967-69 (centred on the 1968 sunspot maximum) was 27% greater than that during the four subsequent years, 1970-73; production in both 1968 and 1969 was higher than in any of the four years 1970-73.

Over much of the Northern Hemisphere, therefore, wheat production appears to be significantly enhanced near sunspot maximum and reduced at sunspot minimum. In parts of the

Southern Hemisphere, however, the opposite is observed. In Argentina, for example, the three years when most wheat was produced were 1954, 1964 (the two sunspot minimum years) and 1963; the average crop for these three years was 50% greater than for the nine years 1965-73. The African wheat crop in 1954 was bigger than in any of the next eight years; it was thus, as in Argentina, bigger than in any year until 1963. In Australia the 1957 (sunspot maximum) crop was smaller than

Researchers at the Appleton Laboratory, in England, say that the present low level of world food reserves may be associated with the decline in solar activity during the approach to the 1974-75 sunspot minimum. Presumably because of their effects on temperature and rainfall, the 11-year and 22-year sunspot cycles appear to influence agricultural productivity in certain parts of the world. Modulations of 10% to 50% of the wheat production in China, the United States and the Soviet Union seem to be correlated with the solar cycles, suggest J. W. King; E. Hurst, A. J. Slater, P. A. Smith and B. Tamkin. The forthcoming World Food Conference in Rome might well wish to discuss implications for global agricultural planning.

in any of the previous eight years; in these eight years an average of 83% more wheat was produced than in 1957.

The facts show that the modulation of wheat production associated with the 11-year sunspot cycle seems to be at least 10% in many parts of the word; in certain countries it may even be greater than 50%. A consequence of this modulation may be the fact that the Soviet wheat crop in 1972 was smaller than that of 1971 which was, in turn, less than that of 1970. It is well known that the 'failure' of the 1972 crop led to massive purchases of wheat from the United States and it may reasonably be suggested, in view of the reduced Soviet crops in the years following the 1957-58 solar maximum, that this failure was associated with the decline of solar activity between the 1968 sunspot maximum and the minimum due in about 1974.

Figure 1 shows that the yield per acre of two crops in Britain appeared to be negatively correlated with sun-

spot number during two complete solar cycles between 1937 and 1957. The cycles seem to have been accompanied by a 10% modulation of the yield per acre but it should be stressed that the relationship between agricultural productivity and the sunspot cycle is not always as clear as Fig. 1 would suggest; some reasons for this are discussed later.

It is not appropriate to describe here the complicated relationships that exist between the solar cycle and the weather in different parts of the world, but some examples of the association between sunspot number, temperature and rainfall are worth mentioning. Figure 2 shows how the rainfall at Fortaleza in Brazil varied during the 'double' or 22-year sunspot cycle; data are plotted for the period 1865-1925 which covered almost three double solar cycles. The rainfall followed the double sunspot cycles closely and the modulation associated with the double cycle amounted to about 35% of the average annual total. P. D. Tyson, as reported in the Johannesburg Star in April this year, observed that the rainfall in parts of South Africa exhibits a pronounced modulation (approximately 25% of average annual total) with a period of about 20 years. We have noticed that this oscillation closely resembles the double sunspot cycle and also that the shorter-period oscillation which Tyson observed in the rainfall over the most southerly part of Africa is in antiphase with the 11-year sunspot cycle.

The rainfall in parts of North America is also strongly influenced by the double sunspot cycle. W. O. Roberts<sup>1</sup> has pointed out that droughts in certain regions of the United States that are important for agricultural purposes occur in the years around every second sunspot minimum. The last drought was centred on the sunspot minimum year 1954 and we believe it is significant that the wheat crop in the United States in that year was 12% smaller than the average for the five previous years. It is also significant that in 1974, when the next drought was to be expected, lack of rain has adversely affected the maize crop in the United States. The wheat figures for 1949-73 suggest that Australian wheat production is also influenced by the double sunspot cycle.

Figure 3 shows how the July temperature in central England varied during the 130 years from 1750–1880; evidently a modulation of about 0.75° C occurred in phase with the double sunspot cycle except for a short period around 1850 when the phase of the temperature variation jumped by half an 11-year cycle. More recently, the growing

season (a measure of temperature) in Scotland has undergone a 10% fluctuation in phase with the 11-year sunspot cycle. The rainfall in England also appears, during the past 80 years at least, to have been influenced by the 11-year cycle. Data from Kew (1891-1930) and Bedford (1931-70) show that the rainfall there underwent a modulation in phase with the 11-year cycle. Averages for periods of four years around the sunspot maxima and four years around the sunspot minima (the extreme year of the solar cycle being the third of the four years in every case) show that at Kew the average rainfall at sunspot maximum (four sunspot maxima, 16 years) was 17% greater than at sunspot minimum (three sunspot minima, 12 years). At Bedford the rainfall at sunspot maximum (16 years) was 16% greater than at sunspot minimum (16 years). It does not seem unreasonable to suggest, since the 11year sunspot cycle appears to modulate both the rainfall and the temperature over Britain, that agricultural productivity may be affected by the solar cycle.

The temperature data shown in Fig. 3 provide evidence of a long-period variation which will be described elsewhere, but it should be pointed out here that the phases of the two longperiod temperature variations (78 years and 181 years) observed in parts of the Northern Hemisphere are such that the maximum temperatures associated with these variations occurred in 1934 and 1922 respectively; the period around 1930 was thus relatively warm. It is well known that the period around 1700 was relatively cold; it is referred to as the "Little Ice Age". Temperature data published by Manley<sup>2</sup> for central England can be used to show that the average length of the growing season during the decade 1691-1700 was about 220 days. Temperatures from Kew for the particularly favourable decade 1931-40 show that the average length of the growing season was approximately 280 days and it is evident (without allowing for the slight temperature difference which exists between central England and Kew) that long-term temperature trends are sufficient to cause the growing season in England to vary by about 60 days, approximately 25%. A return to the conditions which existed around 1700 would obviously result, from an agricultural point of view, in a much less favourable environment than that which has existed during the twentieth century. It is worthy of note that a downward trend (based on farmers' estimates compiled each year for six different crops, and published in The Times, September 2 this year) appears to have occurred recently in British agricultural productivity. The average estimate (with 100%

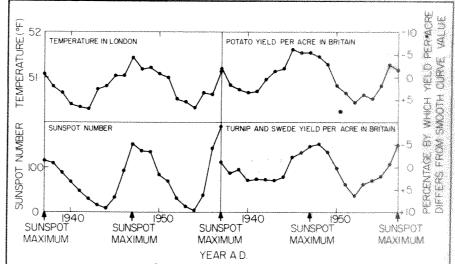
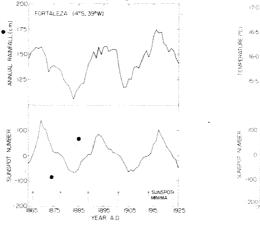
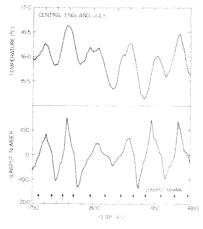


Figure 1 (above) shows variations during two complete sunspot cycles. Top left: 5-point mean of mean annual temperatures at Kew, London. Bottom left: Yearly mean sunspot numbers. Right: Percentage differences between 5-point mean of yearly agricultural yields and the trend obtained by drawing a smooth curve through the 5-point means. Figure 2 (below left): Top: 13-year mean of annual rainfall at Fortaleza, Brazil. Bottom: Yearly mean sunspot numbers, plotted with "even" 11-year cycles inverted in the usual representation of the "double" or 22-year sunspot cycle. Figure 3 (below right): Top: Smoothed mean of the central England July temperatures. Bottom: Yearly mean sunspot numbers, plotted with the even 11-year cycles inverted.





indicating 'full growth') for the years 1964-68 was 94.23%, whereas for the years 1969-74 it was only 91.86%. These figures indicate a decline of 4.6% per decade and, although evidence such as this cannot be used to establish the exact connection between agricultural productivity and long-term climatic trends, it does suggest that the gradual return to colder drier weather which has taken place recently may be having an adverse effect on agricultural output in Britain.

The physical processes through which solar radiation changes associated with the 11-year and 22-year sunspot cycles affect the weather have not yet been identified. At present there is not even a general picture, much less an explanation, of how the climatic effects associated with the solar cycles vary over the Earth. It is already known, however, that the 11-year cycle and the rainfall are positively correlated at some latitudes and negatively correlated at

others; such information could be important for agricultural planning purposes. It is also known that the phase difference between the solar cycle and the rainfall variation at particular locations undergoes sudden jumps of up to 180° but the pattern, if any, of such changes is not known.

Much more work needs to be done to establish the spatial morphology of the various climatic effects associated with the sunspot cycles. It seems reasonable, however, to expect that during the next decade man's knowledge of the effects of the 11-year and 22-year cycles on the weather may advance sufficiently for some of these to be taken into account in global agricultural planning. We thank Mr E. H. Locke and Professor P. D. Tyson for supplying data.

<sup>2</sup> Manley, G., Q. Jl. R. Met. Soc., 100, 389 (1974).

Roberts, W. O., Proc. Symp. on possible relationships between solar activity and meteorological phenomena (NASA, 1974).

# international news

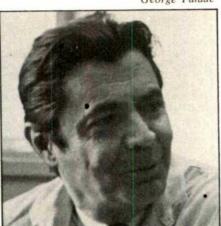
Modern cell biology can now be considered fully established as a rigorous experimental science with the award of this year's Nobel Prize for Medicine and Physiology to Albert Claude, Christian de Duve and George Palade. No other group of biologists has done more than these three laureates to advance our knowledge of the spatial organisation within the cell of diverse biochemical functions—particularly in higher organisms.

To Albert Claude, now 75, it must be particularly gratifying to see this recognition of his pioneering work begun nearly 35 years ago. It was at the Rockefeller Institute (now Rockefeller University) in 1940 that Claude, having recognised the inadequacy of light microscopy and histochemical techniques then available for correlating intracellular structures with their biochemical functions, began to apply electron microscopy to biological material. Also in the early 1940s, he devised the first successful procedure for resolving cellular organelles by the technique of differential centrifugation.

It was the combination of electron microscopy and differential centrifugation of disrupted cells that rapidly laid a foundation for cell biology as we know it. Claude recognised the importance of treating cells with utmost gentleness, both in preserving their structure and during their disruption, in order to gain further insight into the relationship between morphology and function of individual intracellular components. This was soon rewarded by the electron micrographs, taken with K. Porter and E. Fulham which revealed the ultrastructure of cultured animal cells, as well as by the first useful method for biochemically studying isolated mitochondria. The examination of isolated mitochondria in the electron microscope and the analysis of their characteristic enzyme activities in the test tube validated the biochemical studying of isolated subcelfunction in vivo, although there were many who remained sceptical of this approach. Claude soon extended the principle of differential centrifugation to derive a particulate fraction from rat liver cells which were less dense than mitochondria and which he called "microsomes". It was another decade, however, before their intracellular origin and their importance in protein synthesis and secretion, as well as in drug detoxication, were fully realised.

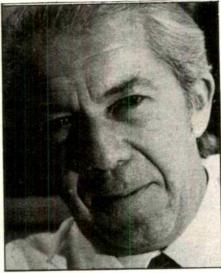
### Nobel Prize 1974

George Palade



Claude's mitochondria isolated from rat liver were heavily contaminated with other subcellular components and the procedure of differential centrifugation was improved in 1948 by his students at the Rockefeller Institute, Hogeboom, Schneider and Palade. Their procedure forms the basis of all the variants used at present in biochemical laboratories throughout the world for preparing active mitochondria and it is hard to overemphasise the impact this development has had on our present concepts of oxidative phosphorylation and biogenesis of mitochondria. Equally im-

Christian de Duve



portant is the fact that the Hogeboom-Schneider-Palade technique formed the basis for the isolation in an active form of other subcellular organelles, to be taken up by de Duve and Palade.

In 1949, Claude returned to his native Belgium to take up the directorship of the Institut Jules Bordet, an event of some importance to Christian de Duve who had then taken up the Chair of Physiological Chemistry at Louvain. De Duve's group was then struggling in an ill-equipped laboratory with the question of the effect of insulin on glycolysis in liver tissue homogenised brutally in a kitchen blender, with "typical disregard of cellular organisation", as de Duve himself expressed a few years later. But Claude's presence was immediately felt when the Louvain group (which included H. Hers and J. Berthet) adopted the swingout ultracentrifuge rotor for investigating the close association of certain groups of hydrolytic enzymes in rat liver. This soon led to the discovery in 1955 of the lysosome, an organelle in which are located all the most important degradative enzymes of the cell, such as phosphatases, nucleases and proteases. De Duve and his collaborators also demonstrated that the lysosomal enzymes existed in a latent state, but could be activated by physical or chemical injury to the cell or to the isolated particle. This observation has led to the publication of several monographs and thousands of papers on the important role of lysosomes as the central element of an "intracellular digestive tract" involved in turnover of cellular components-an indispensable function during cellular differentiation, development of an immune response, carcinogenesis and cell death. Of all the numerous discoveries by these three Nobel laureates, none has more potential value in medicine and pathology than the lysosome. The full realisation of its potential will, however, depend on determining whether altered lysosomal function is a cause or a consequence of the diseased cell.

In the past few years, de Duve, who has occupied a unique dual position at the University of Louvain and at the Rockefeller University, has also been responsible for the discovery of the peroxisome (whose function in vivo is as yet hard to assess) and for the application of the zonal ultra-centrifuge rotor to subcellular fractionation. Perhaps his most outstanding contribu-

UNTIL about 35 years ago the unusual ditions for its formation. In studies of macroscopic thermodynamic and hydroproperties of long-chain macromole- free radical polymerisation, Flory was dynamic properties of a polymer solucules—such as rubber-like elasticity, the first to point out, in 1937, the tion and the average properties and high solution viscosity, abnormally low importance of chain transfer processes interactions of the dissolved chain. The osmotic pressure-seemed so puzzling in controlling molecular weight and to genesis of this work was the now celethat chemists attributed very peculiar show how such processes lead to brated "lattice theory", published in and mysterious intermolecular forces branching and crosslinking. to such subsctances. When it was recognised, however, that covalent struc- Flory has been the study of the physical dynamic properties in polymer softutures comprising thousands of atoms properties of polymers in bulk. These tions from the ideal mixing law. This could be formed, the quantitative include major contributions to rubber work was extended to encompass physicochemical sciences of macro-elasticity theory and experiments, dilute solutions where intra and intermolecular systems could be developed together with the basic understanding molecular excluded-volume effects were without recourse to these mysterious of the swelling of insoluble polymer taken into account. When this work forces. Professor Paul J. Flory of Stan- networks. Professor Flory pioneered was coupled with an analysis of the ford University, this year's recipient of the measurement of the melt viscosity frictional properties of dilute polymer the Nobel Prize for Chemistry, stands of polymers and its interpretation in solution a unified treatment of visforemost among those who have terms of molecular weight, molecular cosity, sedimentation velocity and difdevoted themselves to the development weight distribution, degree of branch- fusion resulted. The well-known Flory of the physical chemistry of high polying and temperature. His paper on the theta temperature, equivalent to the mers. His exacting efforts have pro-statistical thermodynamics of polymer Boyle Point of a real gas, emerged vided the basic conceptual framework crystallisation represents a major con- from this work and prescribed the confor much of polymer science. It is tribution, which clarified an area that ditions in which a polymer solution Flory's distinction to have made had previously been confused. He was behaves ideally. This major developoriginal and fundamental contributions able to show how the crystallisation- ment has allowed for the simple and to virtually every phase of the sub-melting phenomena involving long-chain direct determination of molecular ject. He was a pioneer in establishing molecules fits into the classical frame- weight and of the conformational the basic concept that research in poly- work of a first-order phase transition, properties of chain molecules mer chemistry can be carried out with From this basic concept has come an solution. the same scientific rigour as in other understanding of crystallisation kinebranches of chemistry.

to polymer science consisted of theor- sation, morphology and the properties every area of polymer science. etical and experimental investigations of semicrystalline polymers. These of the principles of condensation poly- ideas were then applied to oriented analysis of complex problems leading to merisation. This pioneering effort in (fibrous) systems with the development essentially simple solutions. The close the application of statistical methods of a quantitative theory of contractility relationship between experiment and to a problem in polymer chemistry was and tension development in the fibrous theory is notable in his work. Experiexact and definitive and was extended proteins and applications to natural mental findings have promoted new to more complicated systems with systems. multifunctional reactants. The result

tics, the recognition of the importance excellence of which continues to this Professor Flory's first contribution of nucleation processes in crystalli- day, shows that it is the cornerstone of

was the concept of an infinite network contributions to the determination of elegant but simply executed experipolymer and a statement of the con- the quantitative relations beween the ments.

1942, which gives an explanation for Another area strongly influenced by the enormous deviations of the thermo-

This description of Flory's work, the

The hallmark of his work is a keen theoretical investigations while the pre-Professor Flory has made major dictions of theory have been tested in L. MANDELKERN

tion to cell biology is his strict adherence to precise quantification and establishing 'balance sheets' for enzymes and other cell components derived by subcellular fractionation, a concept unfamiliar to classical cytologists before Claude's pioneering studies. His elegant use of enzyme markers has led not only to the discovery of the lysosome and the peroxisome but to engendering modern "enzyme cytology".

George Palade, a native of Rumania, was also influenced by Claude when he arrived to work at the Rockefeller Institute in the 1940s. Soon after playing his part in developing a better procedure for isolating mitochondria, Palade innovated techniques for fixation of tissues and subcellular fractions for electron microscopy which have now become standard throughout the world. This enabled him to propose a double membrane model for mitochondria, but by the mid-1950s he turned his attention to "microsomes".

With K. Porter he showed that this subcellular fraction was derived from the endoplasmic reticulum, an intricate network of intracellular membranes and ribosomes. In a fruitful and long lasting collaboration with P. Siekevitz, he provided the first direct confirmation that the ribosome was the site of protein synthesis in the cell. They also showed that active ribosomes existed both as free particles and in a form, predominant in protein secreting cells, in which these are attached to membranes. (Such membranes themselves have since been recognised to be the principal site of metabolism and detoxication of drugs, hormones, carcinogens and so on in the liver.) Students of molecular biology often fail to realise that the foundations for the spectacular success with cell-free preparations from E. coli in understanding the process of translation of genetic information were laid down by the work on rat-liver ribosomes in the laboratories of Palade and Zamecnik.

In the 1960s Palade's group became more and more interested in secretion of proteins and, in a series of elegantly designed and executed experiments involving the pancreas, established that proteins for export from the cell are exclusively synthesised on ribosomes bound to the membranes of the endoplasmic reticulum and not on free polyribosomes. They then described them vectorial passage of the newly snythesised protein into vesicles of "smooth" (ribosome-free) endoplasmic reticular membranes followed by their entry into the Golgi apparatus which would be moving towards the periphery of the cell. Most recently, Palade has turned his attention to the biogenesis of membranes and has proposed the mosaic pattern of arrangement of membrane components.

Finally this year's Nobel Prize highlights an extraordinary success story of research-the modern bio-medical Rockefeller University.

JAMSHED R. TATA

# Slowing energy growth without tears

by Colin Norman, Washington

Environmentalists, opponents of nuclear power and scientists working on energy-saving technologies got some good news recently. A massive study, funded by a grant of £4 million from the Ford Foundation, concluded that it may be possible to meet energy demand in the United States for the next 25 years without enlisting further help from such troublesome sources of power as nuclear reactors, offshore oil, oil shale and strip-mined coal from the Rocky Mountains.

The key to that sanguine prediction can be summed up in one word: conservation. But a good many people have already indicated that they don't believe it, and the Mobil Oil Corporation has published an advertisement in the New York Times to say so.

Called the Energy Policy Project, the study has one over-riding messagethat the United States has a wide variety of energy policy options available and need not be locked into a madcap development of all its domestic energy resources in order to become independent of Middle East oil supplies. In fact, the study argues that such a policy, which was adopted by the Nixon Administration and which has not changed much under President Ford's stewardship, will be inordinately expensive and environmentally destructive.

The most urgent task, the study suggests, is for the government to adopt a set of strong controls to reduce energy demand. Compared with the weak homilies dished up by President Ford during his recent economic message to Congress, when he exhorted his fellow countryman to drive less, wear lapel badges bearing the letters WIN (for Whip Inflation Now) and not to waste food, the Energy Policy Project's recommendations for energy conservation look pretty tough. But, for a country which wastes as much energy as the United States, they shouldn't be too difficult to take.

In essence, the study says that the growth in energy consumption, which has been racing away at about 4.5% in the past few years, can be cut to 2% if the government applies a strong enough hand. Furthermore, contrary to the dire predictions of some economists and industrialists, the study argues that it would be feasible to reduce energy growth to zero by the 1990s without plunging the country into economic stagnation. The four steps which the study says should be taken immediately are:

Energy prices should be allowed to

rise by eliminating subsidies to industry, by imposing pollution taxes on energy producers and by levying extra tariffs on oil imports.

- New cars should meet compulsory fuel economy standards so that the average American car would get 20 miles to the gallon by 1985. At present, the average gas-guzzling behemoth consumes a gallon of petrol every 14 miles, and some of Detroit's creations boast around eight or nine miles per gallon.
- Strong governmental measures should be taken to put a stop to the construction of buildings which have appalling heat losses. This could be done, the study reckons, by raising federally recommended standards and easing credit terms for making energy-saving modifications to existing buildings.
- Federal funding for energy research and development should be channelled more towards energy-saving technologies, and incentive should be given to to industry to apply the technology.

Adoption of those policies, coupled with some good propaganda in support of energy conservation (rather than the ludicrous statements of Nixon earlier this year when he prononuced the energy crisis over), would have the desired effect of slashing rates of energy growth in half, the study reckons. And that would mean that energy demands could be met over the next 10 years without any major new commitment to four controversial sources of supply-increased imports of oil, development of oil shale and coal deposits in the Rocky Mountains (where it is difficult to reclaim the land), nuclear power, and offshore oil drilling along the Atlantic and Pacific coasts and in the Gulf of Alaska.

Even so, energy consumption would increase by a total of about 28% by 1985, with the result that new oil supplies would have to come from the North Slope of Alaska and from secondary and tertiary recovery from existing wells, that coal production must be stepped up from deep mines and from strip mines in areas where reclamation is possible, and that electric power plants which are already being constructed—including nuclear reactors—will have to be brought on line.

But after 1985 it is a different story. If energy use continues to grow even at the reduced rate of 2% a year, at least two of those troublesome sources of supply will have to be opened up. But the point is that by adopting energy conservation measures now, the government can buy itself 10 years in which to choose among the various supply options, on the basis of thorough study of the possible environmental consequences. And, if it is decided that none of them is acceptable, the study says that more stringent conservation

measures could be taken to move the conutry towards zero energy growth by the 1990s.

It should be pointed out, however, that the Energy Policy Project's analysis flies directly in the face of two key assumptions underlying much of industry's-and also the Administration's -approach to energy planning. First, and most important, the study rejects the notion that energy growth and economic growth march hand-in-hand so that cutbacks in energy demand can only be accomplished at the expense of a healthy economy and a high standard of living. And second, the study explicitly states that laissez faire market economics are simply not good enough to regulate energy prices—the government must take an active role in the marketplace.

As for the first notion, according to the Energy Policy Project's director S. David Freemen, who was also energy adviser to Presidents Johnson and Nixon, "energy growth and economic growth can be uncoupled". In fact, the study concludes that cutting energy growth to 2% a year is a more attractive economic option than letting consumption rip at its historic levels. For a start, capital requirements for the energy industry will be \$300,000 million less over the next 25 years than they would be if growth is not curbed. The study also suggests that a gradual reduction in energy growth will actually increase employment because higher prices for energy will tend to substitute labour for energy in industrial processes. Furthermore, apart from the energy industry, industrial outputs will be virtually the same in conditions of reduced energy growth between 1985 and 2000 as they would if recent energy consumption trends were maintained.

As far as zero energy growth is concerned, that would require a strong governmental hand on the tiller and some changes in life style, but again the study predicts that the effects on the economy need not be dire. Such devices as a gradually increasing energy sales tax, expansion of urban mass transit systems, upgrading of rail services and greatly stepped-up research and developmental programmes would be required. The key will be gradual transition to a zero energy growth economy, to avoid major disruptions, and the study says it can be done at the expense of only a very small reduction in the growth of the gross national product.

The study prophetically warns, however, that "merely to discuss 'zero energy growth' is to unleash a torrent of indignant advertising paid for by major industrial interests which benefit from growth in energy consumption".

A Mobil advertisement published on the same day as the EPP report tells its readers that the report recommends substituting bicycles for cars, and that it would "move more than 1,000,000 people a year to new communities where they would work at the jobs planned for them by the government. The advertisement recommends elimination of energy waste (which it neglects to define), urges the development of new energy resources immediately and suggests that some environmental regulations should be relaxed. Without energy growth, Americans would "sacrifice their living standards", the advertisement implies.

Such sentiments can be found within the covers of the report itself, however. Before being published, the report was circulated to members of the Energy Policy Project's Advisory Board—a collection of individuals from industry, academe, environmental organisations and government—and their views appear as an appendix to the study. The nature of their criticisms is fairly well determined by who they represent.

Thus, Michael McClosky, Executive Director of the Sierra Club, takes the study to task for not recommending negative energy growth, and for being too timid in discussing the environmental consequences of high energy growth. Donald C. Burnham, Chairman of Westinghouse Electric Corporation, disagrees with the suggestion that energy growth and economic growth can be uncoupled. The assertions to that end are "totally unsupported by the facts", he says, and the changes proposed "would result in substantial social upheaval, as well as economic stagnation". But the most outspoken criticisms are offered by William P. Tayoulareas, President of Mobil, who says that the report is distorted and offers a recipe for economic disaster.

Be that as it may, will the study have any effect on government policy? The Administration's attitude towards energy conservation, as stated by Mr Ford in his economic message, is that it is clearly a good thing but that at this stage it should be voluntary. Mr Ford said that he hoped consumers and industry would be able to cut back enough to wipe a million barrels of oil a day off the import side of the trade ledger by 1976 and warned that some mandatory controls would have to be applied if voluntary conservation doesn't work. But he specifically rejected a recommendation from his chief energy adviser that a 30% tax should be slapped on petrol, and Congress would be extremely reluctant to take politically sensitive steps to allow energy prices to rise.

The over-riding policy remains as stated often by President Nixon—that domestic energy resources should be developed as rapidly as possible to offset oil imports.

IT was just a year ago that former President Nixon launched Project Independence with a rhetorical flourish and directed the Federal Energy Administration (FEA) to draw up a policy which will make the United States self-sufficient in energy supplies by 1980. Called the Project Independence blueprint, the FEA's plan will be published early in November, but with Nixon's original timetable long ago declared impossible, it represents more a discussion of options than a blueprint for self-sufficiency.

According to conversations with several energy officials and Congressional sources, the study's chief message is that the United States will have to rely on imported oil to meet its energy demands for at least the next decade, and probably for long after that.

Unlike the Ford Foundation study, the FEA blueprint does not suggest that decisions on whether or not to develop controversial domestic energy resources can be put off for a decade, but it does nevertheless put forward some ideas for energy conservation.

First, however, the blueprint suggests that if energy prices remain at present inflated levels, this alone could depress demand and cause the growth in energy consumption in the United States to drop to about 3% a year by 1985. In addition, measures such as mandatory fuel economy standards for new cars, credits for

insulating buildings and incentives for industry to use energy-saving technologies could bring the growth in consumption close to the 2% level recommended in the Ford Foundation study, the FEA reckons.

One reason why the United States is unlikely to stop importing oil in the near future, the study suggests, is that there is only a limited possibility for substituting some of the country's massive deposits of coal for oil. Such a substitution has been vigorously recommended both by former President Nixon and by President Ford, but the FEA analysis points out that only in electricity generation can coal replace oil to a large extent. Consequently, the study predicts that coal consumption will perhaps double by 1985, where as most previous forecasts have suggested that production will be tripled during the next decade. and that oil imports will be needed to meet demand at least until 1985. even with extensive drilling off the Atlantic and Pacific coasts and in the Gulf of Alaska.

The FEA study has some cautionary things to say about strip mining for coal in the arid western regions, suggesting that it will be environmentally destructive and that the coal will in any case contain large quantities of uranium, which could prove to be a health hazard. Similarly, the study pours cold water on the prospects for exploration of oil shale.

# Plans for two more Dutch satellites

from Arie de Kool, Rotterdam

EVEN though the first Dutch satellite, ANS, did not achieve its intended orbit, both astronomers and technicians are in good spirits because the instrument nonetheless works well. This is supposed to prove that Philips and Fokker-VFW may now regard themselves as able space companies. Astronomers are busy reprogramming their computers to make full use of the new orbital parameters.

Such success deserves to be continued—reasons the Dutch National Institute for Aerospace Development, which was established to promote industrial activity in the field. So it proposes two further satellites, both bigger than ANS, and too large to be built within the present decade or launched by a Scout rocket.

'Phase A' preparatory studies and preliminary design studies have been completed for the first satellite, another one devoted to astronomy, in fact an advanced infrared telescope.

The money is supposed to come from the Ministry of Economic Affairs and the Science Ministry. "After all, one should not expect that industry will be prepared to pay for a satellite it did not ask for", said one official of the Economy Department.

The second proposal is for an Earth resources satellite, with the principal objective of plant disease recognition. Most of the money for this one would have to come from the Department for Development Aid. "One of the main problems to be overcome is to convince the developing countries that they do have an interest in cooperation with a neutral country like Holland in this field", said the same government official

A group of scientists has, however, taken issue with these propositions. They feel that other fields of science take priority over space research and they are particularly convinced that remote sensing techniques, if at all useful, can only serve the owners of large monoculture plantations, not the small farmers who should, they say, be considered as the backbone of the developing countries.



the Western hemisphere on a clear most generating stations is only about was implicit in the closure of Tracked morning in October. On the eastern 10 degrees or so above the ambient Hovercraft Ltd early in 1973 and was side of the meridian, and to the right temperature-and so virtually useless foreshadowed in a recent White Paper. of the picture, lies the Great Equatorial for domestic heating purposes. This Maintaining the track on which nothing Building of the Old Royal Observatory, heat could be used if it was rejected has run for over a year costs NRDC Greenwich, its open roof shrouded with from the power station at a higher £128,000 a year. Dismantling costs have protective wrappings. And the structure temperature, but the C.E.G.B. points been quoted at £200,000—but they will which appears to be floating over the out that this would mean a reduction only occur once. trees of Greenwich Park is the tubular in generating capacity which they say steel framework of a new Onion would be very expensive to make up. scrap should have come last week. At Dome, in the process of being hoisted Professor Cassels disputes this point, the very time, barely a mile away at to its place over the pinnacle of He believes that for the 100 MW of the Institution of Electrical Engineers, tarpaulins. The original Onion Dome, generating capacity lost when supplying a big international gathering to discuss made of papier maché over an iron 600 MW of useable heat for a district linear electric motors was listening to framework, was a familiar part of the heating scheme, there would be a 200 Professor Eric Laithwaite (principal Greenwich skyline until it was damaged MW slackening in electricity demand. pioneer and proponent of their applicain the wartime blitz and finally taken So the power lost would not have to tion to high speed land transport) down twenty years ago.

to be thinking of selling its waste heat pipelines and ancillary equipment this for district heating schemes. With five should drop the price of the heat from track at this time may seem doubly major installations, three of which are the Board's estimate of about 240 pence cussed, because rescue seems closer at close to major centres of population, per Gigajoule (pGJ-1) to less than hand than at any time since the British Steel wastes heat equivalent to 200 p GJ<sup>-1</sup>. Although Professor Cassels announcement in Parliament by about 30 million tons of coal each year has not yet completed his detailed Michael Heseltine that the government this energy which would otherwise go district heating becomes competitive. up the flue-or rather down the drainwould be welcome, to say the least.

pool University.

ing schemes are not economical at seal on a government incursion into Colquhoun offer still holds good.

 THE British Steel Corporation is said claim—and even with the experience of linear motors for advanced transport. -worth about £600m. Any return on studies, it is at this point he says that was ceasing to support tracked hover-

If British Steel do go ahead and deliberate destruction by man of a man- option on the track, pending more long market their waste heat they will add made structure. The decision now term decisions about salvaging some of fuel to the discussion between the understood to have been taken at last the five years of development work that Central Electricity Generating Board week's board meeting of the National had centred on Earith. Many people (C.E.G.B.) and Professor J. M. Cassells, Research Development Corporation consider that this work gave Britain a Professor of Nuclear Physics at Liver- (NRDC) to dismantle the mile of hover- lead of several years in the field of train test track at Earith in the fens sophisticated high speed tracked trans-The C.E.G.B. say that district heat- near Cambridge therefore places a sad port. This study continues and the

THE Eastern hemisphere, viewed from present because the heat released from adventurous engineering. This move

It was ironical that the decision to be made up by expensive new oil fired expatiate on his 'magnetic river' degenerating stations-as the C.E.G.B. velopment. An afternoon was devoted to

The move to dismantle the Earith craft. A London firm of consulting engineers, Brian Colquhoun and Part-THERE is something distressing in ners asked NRDC recently for an

# news and views

# Earthquakes predicted

In 1910, H. F. Reid, student of the great San Francisco earthquake and originator of the elastic rebound theory, wrote (in The California Earthquake of April 18, 1906, Carnegie Institution of Washington, 1910): "As strains always precede the rupture and as the strains are sufficiently great to be easily detected before the rupture occurs, in order to foresee tectonic earthquakes it is merely necessary to devise a method of determining the existence of the strains; and the rupture will in general occur in the neighborhood of the line where the strains are greatest, or along an older fault-line where the rock is weakest". Unfortunately, as Brune (Eos, 55, 820; 1974) has recently pointed out, Reid's optimistic assessment, though based on an earthquake model which is generally consistent with modern ideas, contains a flaw which severely constrains the accuracy of any prediction method. For an earthquake is a 'critical limit phenomenon' whose critical limit is influenced by a variety of factors in unpredictable ways. A partial analogy might be the behaviour of a strained wire made of inhomogeneous material. If the load producing the strain is gradually increased the wire will undoubtedly break, but when and where will only become apparent when it is too late. If the single wire is replaced by a bundle of wires (faults) with diverse properties, even the line along which the first break (earthquake) occurs will be unknown in

This inherent difficulty goes some way towards explaining why, 64 years after Reid's statement, reliable earthquake prediction has not yet been achieved. Certainly the lack of success is not for want of effort. Conscious of their extreme vulnerability, the Japanese began to take a serious interest in earthquakes as early as the late nineteenth century when eminent seismologists such as Milne and Oldham were persuaded to make Japan their natural laboratory. More formally, the Imperial Earthquake Investigation Commission was established to encourage seismic studies in the wake of the 1891 Nobi earthquake (magnitude unknown, 7,270 killed); and the 1923 Tokyo earthquake (magnitude 8.3, 99,330 killed) gave the political impetus necessary for the formation of the Earthquake Research Institute at the Tokyo Imperial University. Not all the work which followed was aimed directly at earthquake prediction, although there is no doubt that prediction was the ultimate aim. But in 1961 prediction became a collective research goal when a new group dedicated to that end produced the classic report, Prediction of Earthquakes-Progress to Date and Plans for Further Development. Here the Japanese spelled out their basic philosophy that successful prediction would probably only emerge from long term monitoring of many different aspects of the seismic environment. Specifically, they proposed a 10-year scientific programme involving tide-gauge observations, levelling surveys, repeated triangulation, continuous observation of crustal deformation, micro-earthquake studies and geomagnetic

So far this work has not paid off in any significant way, although it has been responsible for one minor success. During the Matsushiro series of earthquakes which struck central Japan during 1965–1966, a combination of various monitoring data and intelligent guesswork enabled Japanese scientists to give some warning of the particularly violent

activity of April and August 1966. But it is ironic that, in spite of this success and in spite of Japan's long-term commitment to earthquake studies, the current wave of prediction stems from a phenomenon to which they gave comparatively little attention. In 1962, Kondratenko and Nersesov (Trudy Inst. Fiz., Zemli, 25, 130; 1962) discovered that the velocity of crustal P waves increased after moderately large earthquakes in the Tadjikistan region of southern central Asia. They had analysed over 800 travel-time curves from foreshocks and aftershocks of earthquakes with magnitudes 5 or greater and concluded that the average P wave velocity was 5.3 km s<sup>-1</sup> before the main shocks and 6.3 km s<sup>-1</sup> afterwards.

For six years this discovery remained unknown outside the Soviet Union until Savarensky (Tectonophysics, 6, 17; 1968) reviewed the data in English at a conference in Switzerland. Rikitake's (Earth Sci. Rev., 4, 245; 1968) initial response to this revelation was to imply doubt about the validity of the data on the grounds that a P wave velocity change of 15% would correspond to a change in elasticity of 30%. But more results soon followed. Semenov (Izv. Akad. Nauk. SSSR Phys. Solid Earth, 4, 245; 1969), for example, showed that prior to earthquakes in the magnitude range 3-5, the P wave-S wave velocity ratio  $(V_P/V_S)$ decreased by about 6% and then increased again to its normal value just before the main shock. The phenomenon was then taken up by scientists in the United States. A few years later, Aggarwal et al. (Nature, 241, 101; 1973) were able to report that they had discovered VP/Vs decreases of up to 13% preceding New York earthquakes in the magnitude range 1-3. Soon after, Whitcomb et al. (Science, 180, 632; 1973) reported a precursory V<sub>p</sub>/V<sub>s</sub> reduction of 10% for the much larger (magnitude 6.4) San Fernando earthquake of 1971, and showed that the reduction was due largely to changes in  $V_P$ .

By this time a remarkable change of attitude among United States seismologists was apparent. Before the early 1960s most of them had tended to fight shy of any connection with earthquake prediction because of what Oliver (Tectonophysics, 9, 283; 1970) termed its close association with "seers, mystics, fortune-tellers and the like" and the "great publicity . . . given to earthquake predictions based largely on quackery of some sort". But three parallel developments during the 1960s brought earthquake prediction a "great increase in respectability". First was the growth of plate tectonics which gave a new understanding to the role of earthquakes in the global context and thus led to an upsurge of interest in the phenomenon (although by drawing attention to the number of quite different environments in which earthquakes occur, it also seemed to suggest that the prediction problem was even more intractable than had previously been supposed).

The second development, again not directly concerned with prediction, was rather more violent. In 1966, Evans (Mountain Geologist, 3, 23; 1966) concluded that a mysterious series of earthquakes which had been plaguing the Denver, Colorado area for the previous few years was directly related to the injection of fluid into a disposal well at the Rocky Mountain Arsenal some 20 km away. In other words, these were man-made earthquakes; and Evans's explanation of them revived interest in other sources of artificial earthquakes, particularly the shocks thought to be associated with the filling of reservoirs (Carder, in Engineering Geology Case Histories Number 8, Geological Society

of America, 1970). The discovery of man-made earthquakes inevitably raised the question of how they were produced; and this led to a basic understanding of the part played in earthquake mechanisms by fluids. Meanwhile, several workers, beginning with Mogi (Bull. Earthquake Res. Inst., 40, 125; 1962) and continuing with Brace (Tectonophysics, 6, 75; 1968) and Byerlee (J. geophys. Res., 72, 3639; 1967) and others, had been investigating the behaviour of rocks under stress in laboratory conditions, and had come to appreciate the importance of fracturing in rocks in earthquake-prone regions.

All three developments finally came together during 1972-73 in physical models proposed by Nur (*Bull Seismol. Soc. Am.*, **62**, 1217; 1972), Scholz *et al.* (*Science*, **181**, 803; 1973) and Whitcomb *et al.*—models which differ only in

detail. In that of Whitcomb et al., for example, the pores and cracks in the initially unstrained rock are saturated with fluid. As the stress builds up prior to an earthquake the rock increases in volume (dilates), leading to a decrease in pore fluid pressure and ultimately to a situation in which the pore and crack volume exceeds the fluid volume. In this undersaturated state the rock voids partially contain vapour, which reduces the overall bulk modulus,  $V_P$  and hence  $V_P/V_S$ . The initial effect of the dilatancy is thus to delay the onset of the earthquake (dilatancy strengthening); but fluid from outside the dilatant volume gradually flows in, returning the volume to its saturated state, increasing  $V_P/V_S$  and decreasing the fracture strength until the earthquake occurs.

With a promising prediction technique to be developed



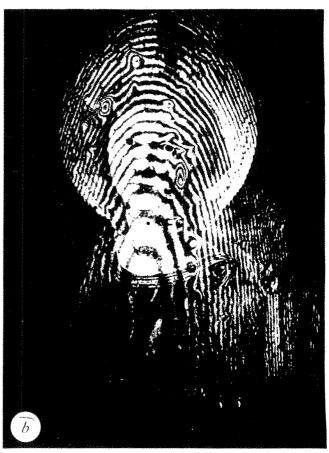


Fig. 1 a, Santa Caterina by Pier Francesco Fiorentina; b, damaged areas indicated by holographic interferometry.

THE detection of incipient damage and deterioration in oil paintings is important for their conservation and restoration. A technique developed by Italian scientists will make this easier in future. S. Amadesi et al. (Applied Optics, 13, 2009; 1974) used holographic interferometry to study paintings on wooden panels. Such pictures have several layers of primer underneath the paint, and these are liable to peel away from the wood. In the early stages the detachment is easier to repair but harder to detect.

Amadesi and his colleagues realised that if a painting were warmed, detached regions would disperse heat at a lower rate than the surrounding areas, so their temperature rise and thermal expansion would be greater.

### Painting holograms

from John Walker

These changes could then be observed by taking two holograms at fiveminute intervals, using a laser. The principle is like superimposing two almost identical photographs: any differences are immediately apparent. The advantage of holograms is that much smaller differences are observable.

In a test run on a laboratory specimen—primed poplar wood—plastic slivers under the primer were easily located. The crucial test was made on the fifteenth century Italian panel painting shown in Fig. 1a. The change in room temperature during

the five minutes between the two holograms was sufficient to indicate some detachments, but localisation was poor. So the painting was warmed to 40° C (not hot enough to cause damage). Many more detachments were then observed, and they were better defined. This is shown in Fig. 1b. The 'contour lines' superimposed on the image are interference fringes, and the kinks indicate detached regions. In undamaged paintings these distortions of the fringe pattern do not occur.

The method can also be applied to frescoes and paintings on canvas. It will be increasingly useful, not only to locate detachments already present, but to monitor undamaged paintings to make sure they stay that way.

and an attractive explanatory physical model to be tested, the expected rapid increase in the number of prediction studies has occurred: at least 10 new reports have appeared during the past three months alone. Mazzella and Morrison (Science, 185, 857; 1974), for example, have gone some way towards confirming the view put forward by Scholz et al. that  $V_P/V_S$  changes should not be the only earthquake precursor attributable to the dilatancy effect. They find that in the middle of April 1973 the resistivity in the vicinity of a strike-slip section of the San Andreas fault south of Hollister (California) suddenly decreased by 10%, subsequently rising again by the end of May to 10-15% above its initial level and dropping back again to that level by 15 June. On 22 June, a magnitude 3.9 earthquake occurred in the region at a depth of 9.5 km. Calculations suggest that the observed variations could have been caused by either a small resistivity change over a large volume or a large change over a small volume; for example, an 80% resistivity change in a region 4 km wide and 2-6 km deep would both explain the data and be physically possible. But either way, as resistivity changes are known to reflect changes in rock porosity and the degree of water saturation, the link with dilatation is not difficult to find.

Although this is the first time that precursory resistivity changes have been associated with strike-slip faulting, the phenomenon is not strictly new in that similar changes of up to 20% were observed by Barsukov (Tectonophysics, 14, 273; 1972) prior to thrust-type earthquakes in the Garm area of the Soviet Union. But what is new is the discovery by Bufe et al. (Geophys. Res. Lett., 1, 221, 1974) that microearthquakes occurring within 10 km of the epicentre of the 1972 Stone Canyon shock (magnitude 4.6) had anomalously large (by 25%) mean focal depths for 60 days preceding the event. Bufe and his colleagues note that it is possible that seismicity really did migrate vertically during this period; but on balance they conclude that the effect is caused by a dilatancy biassing of the foci corresponding to a 10-15% decrease in seismic velocity wtihin a local shallow region.

Moreover, as the Stone Canyon earthquake epicentre fles close to the San Andreas fault within a few kilometres of the area studied by Mazzella and Morrison, this is further evidence of precursors being associated with strike-slip faulting. Last year, doubt about the existence of this phenomenon was expressed by McEvilly and Johnson (Science, 182, 588; 1973) who concluded that there were no P wave changes prior to the 1972 Bear Valley (California) earthquake, although Robinson et al. (Science, 184, 1281; 1974) were later able to refute this suggestion and Wyss and Holcomb (Nature, 245, 139; 1973) had earlier demonstrated the existence of precursors to the strike-slip Matsushiro events. In short, it is now certain that strike-slip faulting is no less susceptible to premonitory indicators than is thrust faulting. This being so, it is clear that the absence of a P wave residual anomaly in central California, now reported by Wyss (Nature, 251, 126; 1974), cannot be attributed to any general lack of precursors to strike-slip tectonism. Instead, therefore, Wyss has interpreted his data to mean that between San Francisco and Parkfield (California) there will be no magnitude 7+ earthquake during the next 7 years and no magnitude 8+ earthquake during the next 25 years. Here is a true prediction, the accuracy of which can only be judged by future events.

But to return to restrospective studies, Wyss and Johnston (J. geophys. Res., 79, 3283; 1974) report that the 1966 Seddon (magnitude 5.8) and Gisborne (magnitude 6.1) earthquakes of New Zealand were associated with P wave changes which began 360 days and 550 days before, respectively. By contrast, McGarr (Science, 185, 1047; 1974) shows that a magnitude 3.8 tremor occurred in South Africa in March 1973 was preceded by no such changes. So is there a problem here, or is the South African shock a

special case? Wang (Nature, 251, 405; 1974) has an ingeneous explanation for contradictions of this sort, based on results obtained last year by Gupta (J. geophys. Res., 78, 6936; 1973). Gupta showed experimentally that when sound waves are transmitted through rock (limestone) containing oriented dilatant microcracks, there is little variation in velocities or velocity ratios in directions parallel to the cracks but large variations perpendicular to them. And apparently unaware of Gupta's work, Anderson et al. (1. geophys. Res., 79, 4011; 1974) have now shown theoretically that fluid-filled ellipsoidal cracks should produce the same effect. Wang's point is thus simple; assuming (and it seems reasonable to do so) that the dilatant cracks lie in a single . direction (probably parallel to the Earth's free surface), whether or not  $V_P$  and  $V_P/V_S$  changes are detected will depend on the direction in which the observed waves are travelling with respect to the cracks. Unfortunately, McGarr, aware of the Anderson et al. article before publication, shows that this cannot be the explanation for the lack of precursors in South Africa. On the other hand, the South African event was not an earthquake but a mining tremor; and so there may after all be good reasons why it can break the rule without destroying the principle.

So where does all this leave earthquake prediction? The enswer seems to be, in quite a healthy state. There are few, if any, genuine contradictions; and the fact that all precursors obey the same time law, with precursor time related logarithmically to earthquake magnitude, strengthens the view that they may be attributed to the same dilatancy mechanism. But routine prediction is not yet possible nor even likely to become so in the near future. The only certain prediction is that in the coming months much will be said on the subject.

Peter J. Smith

# Control of cell growth by the cyclic nucleotide seesaw

NORMAL fibroblasts in tissue culture can exist in two growth states, one of rapid proliferation (growing) and one of relative quiescence (resting). Transition from the resting to the growing state can be produced by a number of stimuli including growth factors present in animal serum, by insulin and by proteases. Once growth is initiated a whole range of events takes place including stimulation of transport of molecules into the cell, increases in protein and RNA synthesis and eventually DNA synthesis. This sequence of events was termed the 'pleiotypic programme' by Tomkins and his collaborators (Nature new Biol., 232, 206; 1971), and a knowledge of how these events are related is fundamental to the understanding of growth control in animal cells.

Since hormones such as insulin and factors such as serum proteins are not thought to enter the cell, Tomkins suggested that they interacted with the cell membrane to modulate the production of chemical mediators that regulate events within the cell. As cyclic AMP has been shown to be the second messenger of many hormones which act at the cell surface, it was only natural to look for changes in the compound in cells stimulated to grow. A substantial drop in the concentration of cyclic AMP was observed within five minutes of the stimulation with serum of the growth of mouse fibroblasts, concomitant with a large increase in the transport of various compounds into the cell: some twenty hours later cell division occurred. If prostaglandin E1 (which raises intracellular cyclic AMP concentrations) was added together with serum some of the transport changes and cell division were inhibited. This led to the

supposition that the fall in cyclic AMP concentrations was in some way a trigger initiating the 'pleiotypic programme'.

But it soon became apparent that falls in cyclic AMP did not invariably accompany induction of growth. When lymphocytes were treated with the mitogen concanavalin A no change was observed in cyclic AMP concentrations but the concentration of the related nucleotide cyclic GMP rose (Goldberg et al., Proc. natn. Acad. Sci., U.S.A., 69, 3024; 1972). This observation led Goldberg to propose that growth was regulated by the balance of cyclic AMP and cyclic GMP in the cell, high cyclic AMP: cyclic GMP ratios being associated with the proliferative state. A recent paper by Rudland (Nature, 251, 417; 1974) documents changes in cyclic AMP and cyclic GMP in a number of cell lines. It shows that non-transformed fibroblasts exhibit large differences in the concentrations of cyclic AMP and cyclic GMP and in the cyclic AMP: cyclic GMP ratios for growing and quiescent cells but that no such difference occurs in transformed fibroblasts. This suggests that there may be fundamental difference in the control of cyclic nucleotide concentrations between normal and transformed cells. In any event low cyclic AMP: cyclic GMP ratios are associated with proliferation, high ratios with quiescence. This concordance of fact with theory is somewhat marred by a recent report that no changes can be detected in cyclic nucleotide concentrations in chick embryo fibroblasts in several different growth states or when the cells are transformed with a number of viruses (Hovi, Keski-Oja, and Vaheri, Cell, 2, 235; 1974). Whether this reflects differences in the mechanism of growth regulation in different cell types or a defect in the hypothesis of growth control by reciprocal changes in cyclic AMP and cyclic GMP concentrations is not clear at the moment.

Correlations of the concentrations of cyclic nucleotides with growth pattern in the cell are unsatisfactory in that they do not prove that the nucleotides actually control growth processes within the cell. Two approaches to this problem have been attempted. One is to add cyclic nucleotides exogenously to attempt to mimic or block growth stimulation by serum, and the other is to isolate simple factors that will specifically alter one of these nucleotides within the cell. In recent series of papers, Rudland and Seifert and collaborators report results of both approaches They reasoned that if an increase in cyclic GMP concentration was a signal for cell division then by adding cyclic GMP exogenously it might be possible to persuade quiescent

cells to divide. The experiment (Nature, 248, 138; 1974) showed that of a number of nucleotides tested only cyclic GMP and its analogues or cyclic IMP were partially effective in promoting division whereas serum can stimulate all the cells to divide. Experiments where serum stimulation is blocked by raising cyclic AMP concentrations have been more successful (Nature, 251, 417; 1974). Almost the entire effect can be overcome using prostaglandin E1, but experiments using high doses of potentially toxic compounds for long periods should be interpreted with care, especially if growth inhibition results.

Using the other approach Rudland took advantage of the purified protein factor isolated by Gospodarowicz from bovine pitditary (Nature, 249, 123; 1974). This factor, termed fibroblast growth factor (FGF), together with hydrocortisone and bovine serum albumin can completely replace serum in its growth promoting activity in certain lines of BALB/c 3T3 cells. What is particularly interesting is that FGF initiates the pleiotypic programme without a significant fall in cyclic AMP concentration (Nature, 250, 791; 1974) -only a large change in cyclic GMP occurs. Furthermore studies on isolated membranes from BALB/c 3T3 cells show that FGF will specifically stimulate guanyl cyclase, leaving adenyl cyclase unaffected. These data strongly suggest that increases in intracellular cyclic GMP concentrations may alone be sufficient to initiate growth without concomitant decreases in cyclic AMP. But it still remains possible that FGF initiates some general membrane change leading to cell growth (such as promoting nutrient uptake) and that cyclic GMP remains merely a correlate of such a change. Only by further dissection of the system by isolation of other factors which affect cell growth in a defined way can the problem be understood.

ROBERT SHIELDS

# Multiple transfer processes in heavy ion reactions

from P. E. Hodgson

EXPERIMENTAL studies of the inelastic scattering of heavy ions have revealed a pronounced oscillatory structure in the angular distributions, and this has been explained as an interference effect between the direct inelastic scattering and one-step transfer inelastic scattering. Such effects are particularly strong when the interacting ions differ only by the transferred nucleon, for example in the reaction

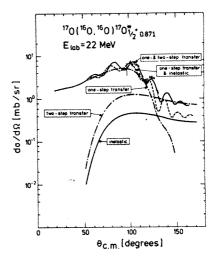


Fig. 1 Differential cross section for the 170(160, 160')170\* (½+, 0.871 MeV) reaction at 22 MeV compared with coupled channel calculations of the direct inelastic, one-step transfer and two-step transfer processes, both individually and combined together.

 $^{17}0(^{16}0, ^{16}0')^{17}0*$ ; so that by the transfer process they effectively exchange their identities (Nature, 248, 735; 1974). Though calculations with this model give a good qualitative account of the data, there remain significant discrepancies, and now Bauer and Wolter (Phys. Lett. 51B, 205; 1974) have shown that these result from the contribution of double transfer processes. Such processes are familiar in the atomic scattering of ions, where electrons are transferred back and forth many times, and some evidence for them has been found in nuclear processes such as the scattering of alpha particles by 'Li (Nature, 246, 14; 1973).

Bauer and Wolter studied the inelastic scattering reaction <sup>17</sup>0(<sup>18</sup>0, <sup>18</sup>0')<sup>17</sup>0\* to the ½ state of <sup>17</sup>0 at 0.871 MeV at several incident energies. They set up a system of coupled wave equations that enabled them to calculate the cross sections of the direct inelastic scattering together with the contributions due to the single and double transfer processes.

The contributions of these processes are compared in Fig. 1 with the experimental cross section for an incident energy of 22 MeV. The direct inelastic process alone gives a smooth angular distribution with its main strength in the backward hemisphere. The onestep transfer alone also gives a smooth distribution but has a much higher cross section and is broadly peaked around 100°. These two processes together interfere to give an oscillatory distribution that agrees qualitatively with the experimental data, but shows a significant phase difference. The twostep transfer alone gives a smooth distribution very similar to the direct inelastic process, but with a higher overall cross section, and when it is combined with the direct inelastic and the one-step transfer it gives a distribution that agrees very well with the experimental data. The inclusion of the two-step process has thus essentially accounted for the phase difference in the previous comparison without this process.

The full calculation with the one and two step transfer processes as well as the direct inelastic scattering was repeated for some other energies and they were seen to give a very good overall account of the change of the cross section with energy. It is likely that the small discrepancies that remain can be accounted for by higher order transfer processes. The calculations also give the cross sections for elastic scattering, and these agree well with the experimental data.

# Flares and spots on dwarf M stars

from R. A. E. Fosbury

PROBABLY the most common selfluminous objects in the Universe are the dwarf M stars: stars which have a mass less than a few tenths that of the Sun but a luminosity which is smaller by a considerably larger factor. Their faintness has, until quite recently, resulted in neglect by observers and theoreticians alike. But this property is an advantage in the study of some curious phenomena which take place in their outer atmospheres. A major flare can increase the brightness of one of these little stars by more than five magnitudes (a factor of one hundred) for a period of a few minutes.

The high kinetic temperatures found in the solar chromosphere and corona are thought to result from the dissipation of a mechanical energy flux which has its origin in the hydrogen convection zone beneath the photosphere. The magneto-acoustic wave modes which are allowed to propagate upwards through the temperature minimum between the photosphere and chromosphere travel through regions of decreasing gas density until they are forced to dissipate their energy as heat through some form of shock. By analogy this form of non-radiative heating is believed to operate in all stars which have a significant convection zone near their surface, that is, those stars which have a spectral type later (cooler) than about F5. Another consequence of the outwardly decreasing density in a stellar atmosphere is the importance of any surface magnetic field in controlling the morphology above the height at which the gas and magnetic pressures are approximately equal. It is the resulting structural inhomogeneity which makes the quantitative study of the chromospheres of stars other than the Sun so difficult.

The existence of stellar chromospheres can be inferred from observations of spectral lines whose profiles show that the temperature increases outwards through part of their region of formation. The best known of these are the famous violet H and K lines of ionised calcium which can be detected in emission in a very large number of late-type stars. Even more useful chromospheric indicators are the analogous lines of ionised magnesium at 280 nm which are now being observed from balloon, rocket and satellite. A subset of the dwarf M stars, known as dMe stars, show also the Balmer lines of hydrogen in emission and it is from this subset that are drawn most, if not all, of the known flare stars.

The flare stars have now provided the clearest observational evidence so far of the existence of starspots. Observations of quasi-periodic brightness variations (outside times of flare activity) by Evans and Bopp at Texas and others have been interpreted as a rotational modulation caused by large dark areas drifting across the visible hemisphere. These spots can cover as much as 50 degrees in latitude and longitude but the actual sizes they derive depend to some extent on the temperatures assigned to them. One might naively think that the sizes of such surface inhomogeneities are fixed in some way by the pressure scale height in the atmosphere, the ratio of this quantity to the stellar radius being roughly constant along the main sequence. D. J. Mullan (Astrophys. J., 186, 1059; 1973) however has put forward the hypothesis that sunspots can be considered as convection cells of some type which extend to the bottom of the solar convection zone. In his latest paper (Astrophys. J., 192, 149; 1974) he extends this idea to the flare stars which, with their position lower down the main sequence, have envelope convection zones with a much greater radial extent. Since the ratio of spot diameter to depth has a minimum value of about two in his theory, he predicts that the diameters of spots should be greater in later spectral types. The predicted sizes are in good agreement with

When the convection zone is confined to a thin shell, as it is in the Sun, the dynamo-generated magnetic field is made up primarily of the higher order multipoles. As the convection zone becomes deeper, however, the lower order multipoles come to dominate until in a fully convective star the field should be mainly dipolar. Mullan suggests that this puts a lower limit on the mass, and hence luminosity, of stars which might be expected to show a rotational

modulation of brightness. If the field is dipolar with rotational and magnetic axes parallel then the spots would be expected to form symmetrically around the poles.

Mullan computes a model for a spot on the flare star YY Gem and estimates a surface field in the spot of about 20 kilogauss. Such large magnetic fields are probably generated by efficient dynamos driven by fast differential rotation. There is some evidence that tidal effects may be partly responsible for triggering the sudden release of energy contained in a magnetic field, resulting in a stellar flare.

### Macrophages eat their way into genetic control of immunity

from Robert Kerbel

MACROPHAGES seem to exist solely for the amusement of immunologists, enabling them to re-discover their importance about every five years. This predictable cycle has been upset by recent events, and one could say it's been a good year—or two—for the macrophage.

To begin with, it appears the macrophage has been restored to its position of being the principal aggressor cell in anti-tumour immunity systems (see for example Eccles and Alexander, Nature, 250, 667; 1974) after being temporarily eased off centre stage by the T cell. Then there was the work of Feldmann and colleagues which assigned a role to macrophages in antibody formation beyond the mere digestion and processing of antigen (Feldmann and Nossal, Transplantn. Rev., 13, 3; 1972); these authors reason that the surface of certain types of macrophage is the main site of T and B cell collaboration, by virtue of the highly cytophilic nature of an antigen-specific factor (IgT, as it is called) liberated by activated T cells, for the surface of macrophages.

But at least these are areas in which macrophages have always been thought to play a part, even if the importance of their role varied from one year to the next. A recent set of papers from the laboratory of J. G. Howard, however, now transports macrophages firmly into an area in which they were never seriously considered previously to have a role-genetic control of the immune response (Howard, Courtenay and Desaymard, Eur. J. Immun., 4, 453; 1974; and Weiner and Bandieri, Eur. J. Immun., 4, 457; 1974). The authors' model involved the use of so-called Biozzi 'high' and 'low' responder mice; these are two lines of mice (Ab/H and Ab/L) which were genetically selected

from outbred Swiss Albino stock for their respective high and low antibody responses to immunisation with sheep red blood cells (Biozzi et al., Annls Immun. Inst. Pasteur, Paris, 115, 965; 1968). Since the original studies with sheep erythrocytes, a number of reports has shown that this separate responsiveness is maintained when using just about any other antigen, including a few which do not require T cells to give a good response. This has gradually led to the view that the genetic difference in the two strains resides at the level of the B lymphocyte, resulting in a relative inability of Ab/L B cells to differentiate and proliferate to antibody-producing cells following contact with antigen.

The results of Howard et al. would seem to put this notion to rest. These authors showed that the antigens levan and dextran B1355, both of which do not require T cells (and both being highly branched polysaccharides), give equivalent antibody responsiveness in the two strains. Furthermore if a haptenic chemical grouping, DNP, was coupled to an antigen which normally gives separate responsiveness (polymerised bacterial flagellin or POL, in this case) then high and low responses to DNP were also obtained, whereas equivalent responses were obtained if the DNP was chemically coupled to levan. This indicates that there is nothing intrinsically defective or different in the ability of B cells with anti-DNP specificity to proliferate and make anti-DNP antibodies in the Ab/L strain. The 'defect' rather is, as the authors say, extrinsic to the lymphocyte population.

What then is the nature of this extrinsic difference? The paper by Weiner and Bandieri suggests that it may reside in the way antigens are handled by macrophages. When peritoneal macrophages were isolated from the two strains and cultured overnight, a striking difference in the morphological appearance of the cells became apparent: the majority of Ab/H macrophages were well spread out, with an irregular cytoplasmic outline and many extensions, whereas most of the Ab/L macrophages assumed a spherical conformation and lacked the many prolongations or extensions so characteristic of macrophages cultured on plastic surfaces. When the authors looked at the way macrophages handled a number of antigens which always give separate responsiveness in the two strains they found these antigens were taken up faster by Ab/L macrophages. Furthermore membranebound antigen disappeared rapidly from Ab/L macrophages whereas it persisted in Ab/Hemacrophages. Intracellular digestion of antigen was more rapid in the Ab/L macrophages and

this may be related to their containing higher levels of lysosomal enzymes.

Thus macrophages from the 'low' responders seemed, if anything, hyperactive. These differences were, however, not observed if the antigen tested with the macrophages was levan. The authors therefore speculate that this hyperactivity might render various antigens more poorly immunogenic in the Ab/L line. Whatever the explanation, it seems that macrophages can express genes which help control antibody responses in these strains.

It also may be of considerable interest to those studying the roles of macrophages in cellular immunity that the Ab/H and Ab/L strains give equivalent responses for virtually all types of such reactions thus far tested, including allograft responses, contact sensitivity to chemicals, and graft-versus-host reactions.

# Species richness in geological time

from Peter D. Moore

A CONCEPT which has received widespread support among ecologists is that the species richness of a stable habitat increases steadily through geological time. The justification for such support can largely be traced back to the work of Southwood (J. Anim. Ecol., 30, 1; 1961) who demonstrated a positive linear correlation between the number of Quaternary subfossil records of various British tree taxa and the number of insect species associated with them. If one assumes that the antiquity and persistence of a tree is reflected by the abundance of its subfossil occurrences, then Southwood's work implies that the longer a tree has been present in these islands in any quantity, the more likely it is that many insect species will be associated with it. The finding suggests that the Quaternary time span has not been sufficient to saturate any tree's resources as an invertebrate microhabitat.

Other data have suggested that the relationship between species richness and time is asymptotic, richness rising rapidly at first, then levelling off. The time scale involved before saturation may be reckoned in decades or centuries rather than millenia. For example, Simberloff and Wilson (*Ecology*, **50**, 278; 1969) exterminated the invertebrate fauna of mangrove islands off the Florida coast and observed that reinvasion occurred in an asymptotic fashion over a relatively short period.

Since Southwood's results are so often used to justify the concept of geological time as a critical factor determining richness, Strong has now attempted to re-analyse Southwood's

data (Proc. natn. Acad. Sci., U.S.A., 71, 2766; 1974). Strong points out a logical inadequacy in Southwood's basic assumption, in that he confounds the antiquity of tree taxa with their past abundance and distribution. To resolve this confusion, Strong plots each of Southwood's tree taxa on a graph relating the logarithm of taxon range (estimated by the number of kilometre square samples in which it is now found) to the logarithm of the number of associated species (using Southwood's figures). The graph shows a strong positive linear correlation. He also found that the distribution of recently introduced taxa about the regression line is not significantly different from that of the native species. Thus recent introductions are saturated with insect species to the same extent as the natives: otherwise they would have fewer insect species than would be expected from a consideration of their area of occupation.

The conclusion from this work is that the area of distribution of a tree is more important as a determinant of its insect species richness than is its antiquity. Evidently the more abundant a tree species is now the more likely it is to have left many fossils in recent Quaternary sediments; since Southwood did not take into consideration the age of subfossil finds, it is not surprising that he found a positive correlation between fossil abundance and insect species richness.

It is unfortunate that Strong did not use more up to date information concerning the insect species richness of certain trees. For example the estimate for larch of about 15 species is far too low, but most values are probably underestimates and the general form of the relationship would probably not have been altered. Strong is wrong, however, on this basis to deny the assertion by May (in Stability and Complexity in Model Ecosystems, Princeton, 1973) that although species richness is well regulated on an ecological time scale the regulated level will increase in geological time. Strong has provided no information on what happens to species richness on a geological time scale except that any change in richness is of little statistical significance in comparison with shortterm changes. The variation of species richness over geological periods remains an area for conjecture.

### Correction

An error was introduced in the final drafting of the article "Oppression or Altruism in Insect Unions?" (Nature 251, 101; 1974). The (female) workers are of course diploid, like their sister queens, and not haploid as stated.

### review article

# Development of general relativity

S. Chandrasekhar\*

This article is based on an invited talk given at the 1974 annual meeting of the American Physical Society in Chicago on the occasion of the award of the Dannie Heineman Prize for Mathematical Physics, February 5, 1974. (The substance of this lecture has been published for private circulation in the Summer 1974 issue of The University of Chicago Magazine.)

In an invited talk at a meeting of this society which Freeman Dyson gave nine years ago<sup>1</sup>, he described the history of the development of the general theory of relativity in the following terms:

"Einstein's theory of gravitation took the world by storm in 1919 for two reasons. In the first place, there was the dramatic prediction of the displacement of star-images by the Sun's gravitational field, the organisation of eclipse expeditions to Brazil and Africa to make the very difficult observations that could test the theory, and the clearly positive outcome of the test. In the second place, there was the appealing philosophical character of the Einstein theory, starting with the postulate of a space-time continuum without any special framework of coordinates to label points of space-time with numbers, so that all the laws of physics should be statements which are true in any possible coordinate system.

To Einstein himself and to many other physicists of the 1920s, including Hermann Weyl and the youthful Pauli, it appeared that this beautiful and successful gravitation theory must be the model for the future development of physics. They expected that further extension of the two principles of Einstein, firstly the representation of physical reality as geometrical in nature, and secondly the insistence on general coordinate invariance, would lead to understanding of electromagnetism and of matter, the chief phenomena that remained outside Einstein's original theory. As is well known, these expectations proved false. The theory of matter and electromagnetism took a totally different direction with the advent of quantum mechanics in 1925. Everybody involved in the development of quantum mechanics forgot rather quickly about gravitation and assumed special coordinate systems without apology. Meanwhile the study of gravitation itself lost interest for physicists because nobody could think of any new observations or experiments. So the theory of gravitation gradually became detached from the rest of physics and was studied only by specialists."

Complementary to Dyson's last statement that the general theory of relativity lost its interest to the physicist because "one could not think of any new observations or experiments" is the statement, which one often hears now, that the renewed interest in general relativity during the 1960s is due to the emergence of new astronomical observations and new planned experiments bearing on the theory. I shall not presume to disagree with these statements. But I must confess that it is not clear to me why some very obvious questions were not asked of general relativity until fairly recently: questions whose

answers could well have contributed to an understanding of the theory itself as distinct from its bearing on possible observations or experiments.

I shall illustrate by some examples the kinds of questions that might have been asked.

### **Orbit of Mercury**

One of the first accomplishments of the general theory of relativity was to show that the trajectory of a test particle in the gravitational field of a central mass is a Kepler ellipse which precesses. The agreement of this predicted precession with that observed for the planet Mercury demonstrated that the theory had successfully met one of Einstein's three crucial tests. The comparison that was made is proper since the approximation of considering Mercury as an infinitesimal test particle in the gravitational field solely of the Sun is an extremely good one. It is well known, however, that in the framework of the Newtonian theory, two finite spherical masses will also describe Kepler ellipses, exactly, about their common centre of mass. On this account, it would be natural to expect that in the framework of general relativity these Kepler ellipses will, in a first non-trivial approximation to the theory, also precess; and, further, that the precession will be given by the same formula that is applicable to the case of an infinitesimal test particle except for the difference that the mass of the central body is replaced by some reduced mass. If these expectations should be justified, then their establishment in the framework of the theory cannot be very difficult. Nevertheless, only twenty years after the founding of the theory was the question asked and its solution attempted by Eddington and Clark, Einstein, Infeld, and Hoffmann, and Fock, independently.

### **Gravitational radiation**

Let me consider a second example. The principal reason why Einstein felt compelled to develop his general theory was, of course, the fact that the Newtonian theory is based on instantaneous action at a distance—a proposition that is contrary to the tenets of special relativity. On the general theory, gravitational forces are propagated with the velocity of light, and as a consequence it would be natural if the theory predicted the emission and propagation of gravitational waves by systems that are nonstationary. Simple considerations of a semiheuristic nature show, as Einstein in fact showed in 1918, that on a linearised version of the theory, gravitational radiation will be emitted by systems that are nonstationary; that such radiation will have a quadrupole character; and finally that the flux of such radiation will be proportional to the square of the third time derivative of the quadrupole moment of the system. Nevertheless, for some forty years serious theoretical doubts were entertained with regard to the reality of the predicted radiation. For example, in the Born-Einstein letters that have recently been published2 there is an undated letter

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from Einstein (there is circumstantial evidence that it was written in 1936) in which he wrote:

"Together with a young collaborator I have arrived at the interesting result that gravitational waves do not exist though they had been assumed a certainty to a first approximation. This shows that the nonlinear general relativistic field equations can tell us more or, rather, limit us more than we have expected."

Again in the last page of Infeld and Plebanski's book on *Motion and Relativity* published in 1961, the statement occurs: "the radiation can be eliminated by a choice of coordinate systems;" and the authors quote Einstein as having said: "We do not have any satisfactory classical theory of radiation. Ritz understood this fact; and he was an intelligent man."

That was in 1961.

To clarify the doubts that were expressed about the reality of the gravitational waves one had only to ask the following question: Do the equations of motion of a system, when carried out systematically to the requisite order, include terms which may be described as expressing radiation-reaction in the precise sense that these terms contribute to a secular decrease of a quantity, which we may call energy, and which is conserved by the system in the immediately lower order fipproximation to the equations of motion?

A remarkable paper<sup>3</sup> published by Andrzej Trautman in 1958 would have resolved this question unambiguously but for an unfortunate oversight. The oversight, when corrected<sup>4</sup>, confirms the expectation. The problem was in fact fully resolved in 1962 by the fundamental investigations of Bondi<sup>5</sup> and Sachs<sup>6</sup> from a different point of view which I shall not elaborate. After their work, no doubts—that is, no theoretical doubts—were entertained with regard to the reality of the predicted gravitational radiation by systems with time-dependent quadrupole moments.

### Schwarzschild

Finally, I shall consider a third example of how one not only did not ask of general relativity questions which would have clarified its meaning but actually argued against its implications.

The solution of Einstein's equations appropriate to the field outside a central spherical distribution of mass was obtained by Karl Schwarzschild in a paper communicated<sup>7</sup> to the Berlin Academy by Einstein on January 13, 1916, just two months after Einstein himself had published the basic equations of his theory. The circumstances under which Schwarzschild derived his now famous solution are not generally known. I shall digress to tell the story, particularly as 1973 was the 100th anniversary of Karl Schwarzschild's birth.

During the spring and summer of 1915, Karl Schwarzschild was with the German army at the Eastern Front-that was during the First World War. Richard Courant once told me that he had met Karl Schwarzschild proceeding to the Eastern Front while he, as a member of the general staff, was with a party retreating from the same front; and Courant said that he was surprised that someone as distinguished as Karl Schwarzschild would be proceeding towards a front which was considered too dangerous for the general staff. In any event, while at the Eastern Front with a small technical staff, Schwarzschild contracted an infectious disease, and he returned to Germany late in the fall of 1915 very ill. He died on May 11, 1916. As Eddington wrote in a notice published later that year8: "Schwarzschild's end is a sad story of long suffering from a terrible illness contracted in the field borne with great courage and patience.'

But it is not generally known that it was during the period of his last illness that Schwarzschild wrote two of his greatest papers—papers that are great by any standard. One of them<sup>9</sup> was devoted to the theory of the Stark effect and the foundations for the interpretation of band spectra on the Bohr-Sommerfeld

theory, and the other was devoted to obtaining the solution of Einstein's equations appropriate to static systems with spherical symmetry.

Now Schwarzschild's solution for the metric external to a spherical source of total inertial mass M, as he gave it, and as it continues to be written, is

$$\mathrm{d}s^2 = -\left(1 - \frac{2GM}{c^2r}\right)c^2\,\mathrm{d}t^2 + \left(1 - \frac{2GM}{c^2r}\right)^{-1}\,\mathrm{d}r^2 + \\ + r^2(\mathrm{d}\theta^2 + \sin^2\theta\,\mathrm{d}\phi^2)$$

Using this metric, Schwarzschild derived the formulae for the precession of the perihelion of Mercury and for the deflection of light, which Einstein had derived earlier by solving the field equations in an approximation beyond the Newtonian.

It will be observed that Schwarzschild's solution has an apparent singularity at

$$r = R_{\rm S} = 2GM/c^2$$

This is the Schwarzschild radius. Schwarzschild himself was puzzled by this singularity, and he resolved the puzzle for himself in this way.

Since Schwarzschild had considered the metric as describing the field external to a static spherically-symmetric distribution of mass, he investigated in a second paper10, communicated for publication a month later (February 24, 1916), the solution of Einstein's equations appropriate to the equilibrium of a static sphere of constant energy density. He showed that the radius of such a configuration can never be less than  $1.125 R_s$ , and he was satisfied with this result since the singularity of the metric at  $r = R_s$  will not be relevant under the circumstances he had contemplated. It was proved by Birkhoff some years later (1923), however, that a spherically symmetric solution of Einstein's vacuum-equation must necessarily be static, and that Schwarzschild's solution will apply even if the source, spherically symmetric, were not static. Schwarzschild's lower limit,  $1.125 R_s$ , is not applicable under non-static conditions, and we are required to examine more carefully whether the surface has any deeper meaning. I turn to this matter, now.

That there is no real singularity of the geometry of spacetime at  $r = R_s$  was realised very soon. In fact, Eddington showed in 1924 that by writing the Schwarzschild solution in a different coordinate system one can avoid even the appearance of a singularity at  $r = R_s$  along future directed time-like trajectories.

The real significance of the surface  $r = R_s$  becomes clear when one examines the family of 'geodesics in the Schwarzschild metric. The discussion is indeed straightforward, but it was explicitly and fully carried out only in 1958 by C. G. Darwin<sup>11</sup>. In retirement, as he was then, Darwin recalls a paper of his written in 1913 on "Orbits of Electrons" in the field of a nucleus and starts his study with the remark that it might be interesting to examine the analogous problem in the framework of general relativity. Darwin further notes that the analysis could easily have been made forty years earlier. So it might have been, but the problem would have appeared too simple to the 'specialists' that Dyson refers to in the statement I quoted at the outset.

The geodesics in the Schwarzschild metric have been discussed and analysed at great length since Darwin's papers. For the present it is sufficient to draw attention to two particular results. The first is that all photon orbits coming from infinity with an impact parameter less than  $3^{3/2} R_s$  must necesarily enter the Schwarzschild sphere and arrive at r=0, that is, as one says now, these geodesics are future incomplete. The second result is that trajectories, null or time-like, not only must end at r=0, but must do so in a finite proper time as measured by a comoving observer. For these reasons the surface  $r=R_s$  is called an *event horizon*.

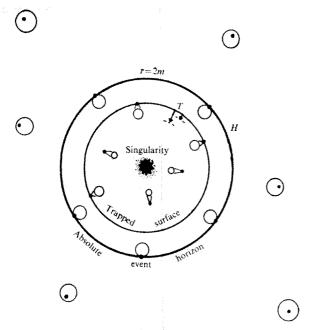


Fig 1 Nature of space-time in the neighbourhood and in the interior of the Schwarzschild radius.

A true picture of the nature of space-time in the neighbourhood and in the interior of the Schwarzschild radius is obtained in the following manner of illustration due to Roger Penrose. In this picture (Fig. 1), the wave fronts of light signals emanating at various points at a slightly later time are shown. At large distances from the event horizon, the points lie at the centres of the respective wave fronts. At smaller distances the wave fronts are displaced by the strong gravitational field present. The wave fronts emanating from points on the event horizon touch the event horizon on the inside and cannot emerge. Inside the event horizon the centre of emission is no longer even inside the wave front; consequently, once inside the Schwarzschild sphere, one cannot communicate with the world outside; and moreover, one would inexorably be propelled towards the centre: not all the King's horses nor all the King's men can prevent it from happening. It should be noted that to deduce all these properties, one has only to draw the light cones at various points—a construction that is entirely elementary.

In essence the significance of the Schwarzschild surface at  $r = R_s$  must have been known to Eddington, certainly by the early 1930s; but nevertheless he would not accept a conclusion which appeared inevitable.

That there is an upper limit to the mass of degenerate stellar configuration had been established<sup>12</sup> by early 1931. Its significance for the final stages of the evolution of stars was fully appreciated at that time as the following extract from what was written at that time shows13:

"The life history of a star of small mass must be essentially different from the life history of a star of large mass. For a star of small mass the natural white-dwarf stage is an initial step towards complete extinction. A star of large mass cannot pass into the white-dwarf stage and one is left speculating on other possibilities."

And Eddington fully realised what this conclusion implied. He stated14:

"The star apparently has to go on radiating and radiating and contracting and contracting until, I suppose, it gets down to a few kilometres radius when gravity becomes strong enough to hold the radiation and the star can at last find peace.'

This remark makes it clear that Eddington realised that the

existence of an upper limit to white-dwarf configurations inevitably requires that in the course of evolution of at least some of the massive stars, black holes—as we now call them must form. But he would not accept the conclusion. For he continued to say:

"I felt driven to the conclusion that this was almost a reducti ad absurdum of the relativistic degeneracy formula. Various accidents may intervene to save the star, but I want more protection than that. I think that there should be a law of nature to prevent the star from behaving in this absurd way."

Indeed, since he considered the conclusion derived from the theory of Fermi degeneracy of electrons, allowing for the effects of special relativity, as leading to a reductio ad absurdum, he modified the relativistic-degeneracy formula so that finite equilibrium states will be possible for all masses.

It is interesting to speculate why Eddington who was one of the earliest and staunchest supporters of general relativity should have found the conclusion that black holes may occur in nature so unacceptable.

### Natural home

The three examples I have described at some length all pre-date the 1960s and are in the context of questions that might have been asked of general relativity, questions whose answers would have contributed to our understanding of general relativity. It will be noticed that the questions one might have asked are precisely questions that have been fully answered during the 1960s in the context of relativistic astrophysics. This is not accidental. In a real sense astronomy is the natural home for general relativity. It has to be so, for the general theory of relativity is a theory of gravity; and in what context, except in the context of astronomy, can one hope to observe manifestations of phenomena derived from strong gravitational fields? In other words, the right physical questions one can ask of general relativity are necessarily in the context of astronomical possibilities.

By saying that astronomy is the natural home for the general theory of relativity, I am envisaging a role for theory in astronomy which is not generally accepted. In my judgment, theory has a double role to play in astronomy: the common one of providing interpretations for observed phenomena; and the uncommon one of providing for astronomy the kind of basis which experiments provide for physics. The latter role is largely unrecognised and largely not practised. But one would certainly recognise this role if one would only stop and realise that unless one can be certain of what one might observe in well defined astronomical contexts, that is, under certain well defined conditions, one could never be sure of any inference that one may draw from observations.

Although I do not wish to go so far, there is an element of truth in an aphorism of Eddington's:

"You cannot believe in astronomical observations before they are confirmed by theory."

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# articles

### Remnant arcs and marginal basins in the Cainozoic development of the Mediterranean

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Migrations of trench arcs into older marginal basins determined the evolution of the Mediterranean region after the Cretaceous-Eocene closure of the original ocean. Remnant arcs, which now form Corsica, Sardinia and the Balearic Islands separate the Miocene, Pliocene and Recent sea basins. Some remnant arcs such as the Apuseni Mountains separate ensialic basins; others such as the Kabylides, are welded at the fore continental

THE present tectonic structure of the Mediterranean area can only be understood if the geological and structural evolution during the past 100 Myr is considered. Although plate tectonic processes can explain neatly the development of the region there are several problems.

First, the evidence that some basins have opened seems to constrast with a general contraction of this region.

Second, there is a divergence between the continuous alignment of post-Miocene structural units and older discontinuous dispersed elements. That can be explained by assuming a very strong horizontal migration driven by recent arcs, which agrees with the greater length and more pronounced curvatures of the more recent fold belts.

Third, studies of the systematic migration of folded and magmatic arcs in the area suggests that the apparent bipolarity of the main structures is a result of temporally distinct processes1. Coeval symmetric double arc complexes cannot alone be responsible<sup>2</sup>.

• Finally, geophysical and geological data<sup>3-6</sup> suggest that the most recognisable basins (the Mediterranean and surrounding components) spread after a phase of general closure of the ocean. That contradicts the simple idea that the Mediterranean Sea is all that remains of an ocean which has been closing since the Mesozoic<sup>7</sup>.

These problems can be studied using models<sup>8-13</sup> of the development of folded, magmatic arcs and back-arc zones at consuming plate boundaries in the circum-Pacific area.

Geodynamic model for the Mediterranean region

The peculiar character of the structural framework of the Mediterranean area is evidenced by the orientation and winding of the folded arcs, many of which are often connected with others of opposite polarity (Fig. 1). Because the original Cretaceous-Eocene arcs are of opposite polarities, two main zones can be distinguished: the Betic-Alpine zone and the Dinaric-Hellenic zone. The original alignments of these palaeoarcs have been disrupted and in places the arcs have been shifted considerably by successive phases

of contraction during the Neogene. The accompanying shift of former internal zones seems to correspond broadly to reverse polarities of Neogene arc trench systems, and also to a maximum expansion of the marginal basins behind the arcs. The alignment of oceanic scars marked by mafic and ultramafic masses (Fig. 1) shows the same pattern. In spite of their structural relationships with other units the origina location of these ophiolite scars can be identified fairly easily with the two Betic-Alpine and Dinaric-Hellenic belt: which were the true oceanic geosuture. It is debatable whether the two principal alignments of the ophiolitic out crops correspond to one or two oceanic areas14 (tentatively: northernmost Pyreneean-Valaisan-Vardar alignment and Betic-Calabrian-Corsica-Ligurian-Piemon southernmost tais-Serbian-Subpelagonian alignment). The ophiolites which are in any case relicts of a pre-Cretaceous (generally Jurassic) ocean floor indicate a major oceanic closure during the Cretaceous-Eocene (Abbate, E., et al., un published).

The evidence that general contraction of the crust continued after the Eocene suggests that most post-Eocene subduction, and consequently the widest arc migrations must be related to the subduction of the floors of Cretaceous-Eocene marginal basins rather than to the subduction of the original ocean floor. That is how both the western central Mediterranean, and to a lesser degree the Carpathian area, evolved tectonically.

The migration of the Alpine and Hellenic arcs seems however, to have developed with a fairly constant polarity since the Cretaceous. So, if the present structure is considered, at least four main zones can be distinguished (from east to west: Hellenic arcs; Carpathian arcs; Alpine arcs: and Apennine-Tell-Rif arcs).

The first two of these arc systems originated from former Dianaric-Hellenic alignment and the others originated from the former Betic-Alpine alignment.

### Hellenic and Carpathian arcs

Behind the Hellenic trenches there is a series of magmatic arcs which, together with the Aegean Basin constitute a well known, active, marginal basin are trench system. They are recognisable as well conserved relicts of older folded and magmatic arcs and although they all show the same polarity they become progressively older northwards.

In the Hellenic area, fore arc contraction up until the Eocene occurred by the closure of the original oceanic floor. We think that the Balkans, with an opposite Cretaceous-Eocene polarity, can be considered as the coeval

back-arc thrust Belt of the Hellenic arcs18.

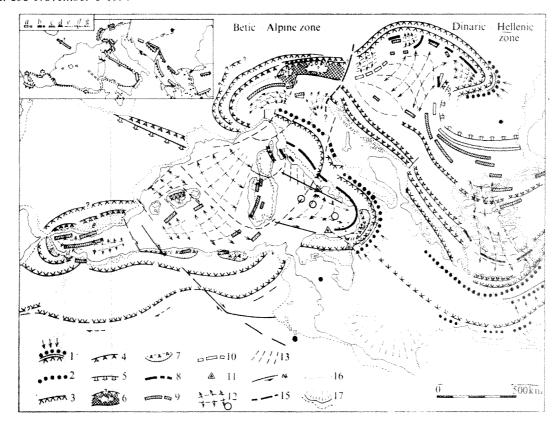


Fig. 1 Geodynamic scheme of the Mediterranean region according to plate tectonic models. 1, Arctrench systems with active subduction; 2, post-Miocene trench alignments; 3, main Miocene and post-Miocene folded arcs; 4, main Cretaceous-Eocene folded arcs; 5, possible Cretaceous-Eocene back-arc thrust belts; 6, Austro-alpine overthrust; 7, microsialic elements of the same Cretaceous-Eocene Betic-Alpine alignment of the Tellian-Apennine arcs. The original 'mother' continent of these sialic fragments, Austro-alpine<sup>6</sup> included, could be the palaeo-African plate (see text): a, Calabrides; b, and c Kabylides; d, Sebtides and Ghomarides: e, Alpujarrides. 8, Post-Tortonian magmatic arcs; 9, Oligo(?)-Miocene magmatic arcs; 10, Cretaceous-Eocene magmatic arcs (relicts); 11, magmatism mainly connected with shear lines; 12, back-arc spread marginal basins with basic magmatism on the floor; 13, pre-spread back-arc distended areas; 14, transform and transcurrent faults; 15, 'Lijubljana-Wien' line separating the Betic-Alpine zone from the Dinaric-Hellenic zone (Cretaceous trench-trench sinistral transform fault and Neogene western limit of the Pannonian and Wien basins); 16, negative gravity anomalies; 17, continental rises and shelves. Inset, correlation and shift of main Mesozoic mafic and ultramafic outcrops: a, alignment of Mesozoic ophiolites considered as elements which have migrated from a southernmost oceanic geosuture; b, ophiolitic elements which have migrated from a northermost ocean geosuture; c, original polarity of the Cretaceous-Eocene geosutures; d, successive polarities during the migration of the reversed arc-trench system; e, shear lines; f, zone (Colantoni, P., et al., unpublished); g, correlation line.

Post-Eocene contraction phases of the arcs developed on the external side, where deep mantle subduction could correspond to the shallow overthrusts of a very thin sialic cover (for instance, the Mediterranean Ridge). Anyway, the type of crust which characterised the floor of the present eastern Mediterranean is still questionable<sup>4,16</sup>.

Instead, the Carpathian area seems to have developed at the expense of the marginal basin behind the Dinarids, with a general inversion of arc polarity occurring after Dinaric welding during the Eocene. This Neogene contraction in its turn determined the spreading of new first order marginal basins (Pannonian Basin) and second order marginal basins (Wien Basin, Transylvanian Basin, and so on). Active subduction is still evidently associated with the extreme eastern Carpathians<sup>17</sup>.

The Apuseni Mountains can also be considered as a remnant arc containing the more internal parts of the inner Dinarids.

### Alpine and Apennine-Tell-Rif arcs

The western Mediterranean was formed from Neogene and Quaternary marginal basins<sup>6,18,19</sup> developed behind the Apennine-Tell-Rif arcs as they migrated southwards and eastwards. The marginal basins, which are of different

stages of maturity are (Fig. 1): the 'Alboran Basin' formed behind the Rif Arc; the first order 'Valencia Basin' formed behind the remnant Baleares Arc; the second order 'Balearic Basin' formed behind the Tell Arc; the first order 'Ligurian Basin' formed behind the remnant Corsica-Sardinia arcs; the second order 'North Tyrrhenian Basin' formed behind the Northern Apennines arc; and the second order 'South Tyrrhenian Basin' formed behind the Calabrian Arc, which is still active.

It is possible to construct a sequence of palinspastic sketch maps to show the possible tectonic evolution of these systems (Fig. 2). There were three phases of Betic-Alpine contraction from the Cretaceous to the Eocene (Fig. 2A, B, and C). The palaeo-European continental margin (of the Atlantic type) includes the sialic fragment of the Balearic Islands in continuity with Iberian sial, and also the Corsica and Sardinia microcontinents (with part of the palaeo-Calabrian sialic substratum) in continuity with the southern French margin. The palaeo-African continental margin (of the Pacific type) is characterised by a festoon of arcs which were migrating northwards together with some detached fragments of African sial. The external ocean floor (Fig. 2A, B) can be correlated with the high pressure and temperature metamorphosed ophiolite suites

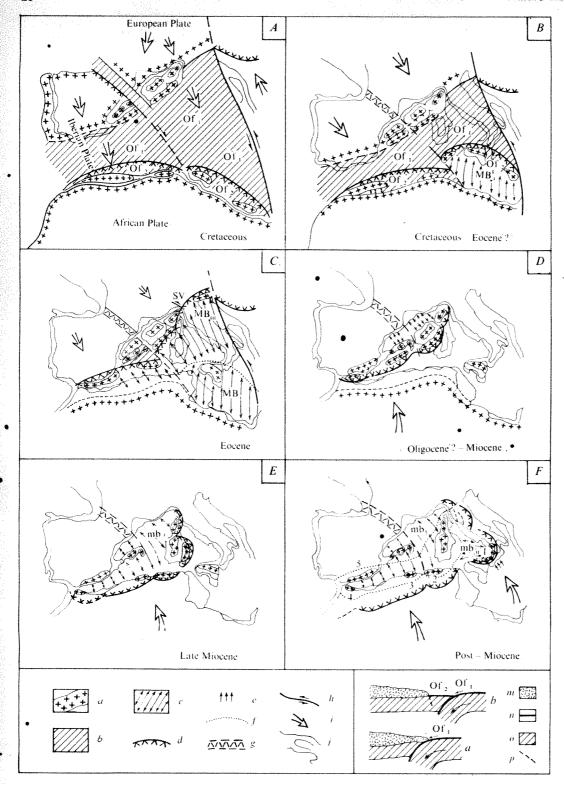


Fig. 2 Hypothetical geodynamic development of the western Mediterranean region. A-C, Creta-ceous-Eocene phases of oceanic contraction with development northward Betic-Alpine belt up to the arc-continent geosuture, with maximum opening of back-arc marginal basins; D-F, Neogene phases of reversed migration of the southward and eastward Rif-Tell-Apennine arc-trench systems up until the present, with closure of former external marginal basins and opening of new back-are basins (the actual western Medibasins, see Continental terranean text). a, Continental crust; b, Mesozoic ocean floor; c, marginal basins; d, folded arcs, showing polarity; e, active subduction areas; f, previous position of palaeoarcs for reference; g, Pyre-naic geosuture; h, trans-form faults; i, possible movements of palaeoplates relative to the deep mantle<sup>31</sup>; j, present geographic contours for reference; MB<sub>I</sub> and MB<sub>II</sub>, first and second order Alpine marginal basins; mb<sub>I</sub> and mb<sub>II</sub>, first and second order Apennine marginal basins. 1, Calabrides; 2 and 3, Kabylides; 4, Sebtides and Ghoma-rides; 5, Alpujarrides. rides; 5, Alpujarrides. Of-1, High pressure and Alpujarrides. temperature metamorphic ophiolites suites belonging to the fore-trench oceanic floor (Schistes Lustrès p.p.); Of-2, low pressure and temperature metamorphic ophiolite suites of the back-trench oceanic floor ('Ligurian' units). SV, Sestri-Voltaggio line; m, continental crust; n, oceanic crust; o, upper mantle; p, pos-sible obduction occurr-

of the Schistes Lustrès complex<sup>20,21</sup>. The internal ocean floor Fig. 2A, B) can be correlated with low pressure and temperature metamorphic ophiolite suites of the Ligurids complex<sup>22</sup>.

The arc-continent collision (Fig. 2C) corresponds to the main geosuture phase recorded by the Betic-Alpine belt. The Pyrenean suture (Fig. 2B) has been indicated tentatively at the Cretaceous-Eocene boundary<sup>23</sup>.

That was followed by an inversion phase (Fig. 2D) when new arcs, with reverse polarity, became detached and started migrating southwards and eastwards. After the Miocene it seems convenient to speak of a pseudo-Pacific and pseudo-Atlantic type for the European and the African continental margins, respectively, because the original Mesozoic ocean had been practically destroyed during the

Cretaceous-Eocene subduction and had been replaced by a system of marginal basins. The continuing migration of the arc trench systems into older Betic-Alpine marginal basins, caused the opening of new marginal basins in the new back-arc area. These Miocene and post-Miocene marginal basins constitute the present western Mediterranean (Fig. 2E and F).

In this context, Corsica, Sardinia and perhaps the Balearic Islands can be considered as interbasins remnant arcs which more or less had their south-eastern sialic margins 'amputated'. These minor fragments were involved in the subsequent arc migration, and in places contained elements of Betic-Alpine scars and covers (for example, the Calabrian Arc).

Thus, the correlation of Calabrides, Kabylides, Sebtides, Ghomarides, Alpujarrides and Malaguides24,26, can be carried up to the Austroalpine overthrust (Fig. 1) and they can all be considered as African elements. In summary, they first welded against the European borders during the Eocene (Fig. 2C) and were then either partially bounced back again on to the late Miocene and post-Miocene African border or are still migrating south-eastwards with the Calabrian Arc (Fig. 2F).

### Unexplained features

Among the many items requiring explanation is the palaeogeography of branches of the Mesozoic ocean. Was, for instance the Pyrenean oceanic channel continuous with the eventual northern Mesozoic oceanic branch? If so, were the opening and closure of the two oceanic branches contemporaneous? Another point needing clarification is the size and width of oceanic branches which have total estimated dimensions, when assembled, of more than 1,000 km<sup>1</sup>

The relicts of the Cretaceous-Eocene magmatic arcs are scarcely recognisable in the western Mediterranean region possibly because of the general inversion of subduction directions and the structural counterthrusts in Oligo(?)-Miocene times.

The external zones of the arcs also pose a problem: both the folding of the outermost covers, and the detachment and shear of the foreland carbonatic platforms27 need to be investigated. For the first point we speculate that the well known gliding tectonics could be related, to a certain degree, to the last vertical effect of the previous active subduction. For the second point, a palinspastic reconstruction is necessary. This must take into account synmigrating sedimentation and a progressive decrease and fragmentation of the original carbonate shelves.

Finally, one of the main geological queries concerns the origin of the Mediterranean evaporites, both buried in the present Mediterranean basins and outcropping along many external arcs28

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## New RNA polymerase from *Bacillus subtilis* infected with phage PBS2

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Bacillus subtilis infected with bacteriophage PBS2 contains a new rifampicin-resistant RNA polymerase that is apparently composed of four different polypeptides.

THE growth of phage such as T4 (ref. 1), SP01 (ref. 2) and T7 (ref. 3) requires the DNA-dependent RNA polymerase of the host bacteria. Studies with the drug rifampicin, an inhibitor of bacterial RNA polymerases, have indicated that transcription by coliphage T4 and Bacillus subtilis phage SP01 requires the rifampicin-sensitive component of the host RNA polymerase throughout the lytic cycle<sup>1,2</sup>. On the other hand, the growth of coliphage T7 is only sensitive to rifampicin during the first few minutes of infection3 since this phage elaborates a new drugresistant RNA polymerase that directs RNA synthesis late in the lytic cycle<sup>4</sup>. Nevertheless, the host RNA polymerase is initially required for the synthesis of the new T7 enzyme.

PBS2 is a large B. subtilis phage whose DNA genome contains uracil in place of thymine<sup>5</sup>. In contrast to the growth of phage T4, SP01 and T7, growth and transcription by PBS2 are resistant to rifampicin throughout the lytic cycle even if the drug is administered to the host bacteria several minutes before infection<sup>6,7</sup> (T. Fox and S. Clark, unpublished results). A possible explanation for this finding is that when it infects B. subtilis PBS2 injects a new drug-resistant RNA polymerase along with the phage genome. Another possibility is that PBS2 utilises a previously undetected rifampicin-resistant RNA polymerase of the host bacteria. To investigate these possibilities we have searched for a rifampicin-resistant RNA polymerase in extracts of PBS2-infected bacteria.

### Isolation of enzyme

A new RNA polymerase that copies PBS2 DNA in the presence of rifampicin was first detected in crude extracts of PBS2-infected B. subtilis (fraction 1, Table 1). This drug-resistant enzyme was purified partially by ammonium sulphate fractionation (fraction 2) and DEAE-cellulose chromatography (fraction 3). (The enzyme activity at this stage of purification depends completely on added PBS2 DNA.) The enzyme was next separated from B. subtilis RNA polymerase by phosphocellulose chromatography. Host and rifampicin-resistant enzymes were eluted by buffers containing 0.4 M and 0.55 M KCl respectively.

Table 1 Summary of	purifi	ication of	PBS2	RNA po	lymerase
Fraction	$\frac{A_{280}}{A_{260}}$	Total protein	Total activity	Specific activity	Re- covery
(1) High speed		(mg)	(U)	(U mg <sup>21</sup> )	(%)
supernatant (2) (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.53	2890	114	0.04	100
precipitate (3) Pooled DEAE-	0.48	2750	162	0.06	•142
cellulose enzyme (4) Pooled phospho-	1.2	800	186	0.23	163
cellulose enzyme (5) Pooled DEAE- Sephadex	1.4	6	160	26.7	140 •
enzyme (6) Pooled glycerol	*****	0.48	102	212	89
gradient enzyme*	******	0.12	54	450	47

B. subtilis 3610 growing at 32° C in 121A medium<sup>8</sup> was infected at a multiplicity of three and collected between 40 and 60 min after infection. The enzyme was purified from 60 g of infected cells. PSB2 RNA polymerase activity was assayed in the presence of 20 μg ml<sup>-1</sup> rifampicin with 4 μg PBS2 DNA as a template. The reaction mixture was as previously described except that the radioactive nucleotide was <sup>3</sup>H-UTP (200 mCi mmol<sup>-1</sup>) and KCl was omitted. One unit enzyme incorporates 1 nmol of <sup>3</sup>H-UMP in 10 min at 37° C. Sonication and high speed centrifugation were as described previously <sup>10</sup>. The enzyme was precipitated from the high speed supernatant fluid by addition of ammonium sulphate to 60% saturation and collected by centrifugation at 100,000g for 1 h. The pellet was resuspended in 100 ml buffer C (0.05 M Tris-HCl, pH 8, 0.1 mM EDTA, 0.1 mM dithiothreitol, 10% v/v glycerol) containing 0.1 M KCl and dialysed against the same buffer. The dialysed enzyme was diluted twofold and applied to a 500 ml DEAE-cellulose column (5.7 × 20 cm) equilibrated with buffer C containing 0.24 M KCl. This fraction was applied to a 15 ml phosphocellulose column (1.9 × 5.1 cm) equilibrated with buffer C containing 0.24 M KCl. This fraction was applied to a 15 ml phosphocellulose column (1.9 × 5.1 cm) equilibrated with buffer C containing 0.24 M KCl. This fraction was applied to a 15 ml phosphocellulose column (1.9 × 5.1 cm) equilibrated with buffer C containing 0.24 M KCl. The polymerase was then eluted with buffer C containing 0.55 M KCl. The phosphocellulose purified enzyme was dialysed against buffer C containing 0.01 M MgCl<sub>2</sub> and 0.1 M KCl and further purified by DEAE-Sephadex chromatography and glycerol gradient centrifugation as described in the legends to Figs. 1 and 2.

\*The fraction 6 activity and recovery have been normalised assuming that all the fraction 5 enzyme had been applied to the glycerol gradient (see legend to Fig. 2).

The latter eluate (fraction 4) was applied to a DEAE-Sephadex column and eluted with a linear gradient of 0.1-0.5 M KCl (Fig. 1). The rifampicin-resistant activity eluted as a single peak at about 0.33 M KCl (fraction 5). Alternate fractions from the DEAE-Sephadex gradients were analysed by sodium dodecyl sulphate (SDS) polyacrylamide slab gel electrophoresis (Fig. 1). Fractions displaying the peak of enzyme activity contained four major polypeptides of 80,000 (I), 76,000 (II), 58,000 (III), and 48,000 (IV) daltons in addition to several other polypeptides in lesser amounts. (Molecular weights were determined by SDS disc gel electrophoresis in phosphate buffer.) As a final purification step, rifampicin-resistant enzyme was subjected to zone centrifugation through a linear gradient of 10-30 % (v/v) glycerol. The enzyme (fraction 6) showed a single peak of activity at 11S, coinciding with a peak of protein and indicating high purity (Fig. 2). SDS polyacrylamide slab gel electrophoresis of the glycerol gradient fractions showed that only the four major polypeptides (I, II, III and IV) of the DEAE-Sephadex-purified

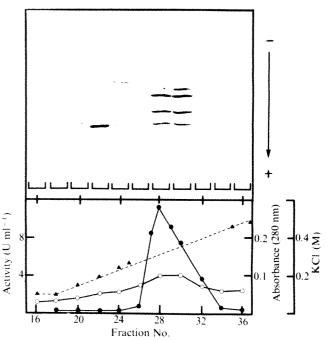


Fig. 1 DEAE-Sephadex column profile. Fraction 4 PBS2 RNA polymerase (6 mg) was applied to a 2.5 ml DEAE-Sephadex column (0.8  $\times$  4.8 cm) equilibrated with buffer C containing 0.1 M KCl and 0.01 M MgCl<sub>2</sub>. The column was washed with 20 ml of the same buffer. The enzyme was then eluted with a linear gradient of 0.1 to 0.5 M KCl in buffer C containing 0.01 M MgCl<sub>2</sub> and 5% (v/v) glycerol. Fractions of 2.4 ml were collected and 25 µl was assayed as described in the legend to Table 1 with 3'H-UTP (100 mCi mmol<sup>-1</sup>) ( $\blacksquare$ ). Alternate fractions were subjected to SDS polyacrylamide gel electrophoresis (8% w/v acrylamide)<sup>11</sup>.  $\bigcirc$ , Absorbance at 280 nm.  $\blacktriangle$ , Molarity of KCl.

enzyme sedimented coincident with the rifampicin-resistant activity. A densitomer scan of a stained SDS disc gel of the glycerol gradient-purified enzyme indicated that the stoichiometry of polypeptides I, II, III and IV was 1.2, 1.3, 1.0 and 0.9 respectively. None of these polypeptides corresponded to the known subunits ( $\beta\beta'\alpha\sigma$ ) of B. subtilis RNA polymerase (Fig. 3).

Glycerol gradient analysis indicated that the rifampicinresistant RNA polymerase is a large enzyme that appears to contain four subunits. Moreover, a molecular weight of 262,000 for a tetrameric enzyme containing all four of the polypeptides is consistent with a sedimentation coefficient of 11S. Proof of this subunit structure, however, will require reconstitution of the enzyme from the separated polypeptides. It cannot be excluded, for example, that the rifampicin-resistant RNA polymerase consists of an aggregate of just one of the four polypeptides and the other three polypeptides are contaminants that happen to copurify with the enzyme even after a greater than 10,000-fold purification (Table 1).

In a preliminary experiment rifampicin-resistant RNA polymerase was also purified partially from PBS2-infected *B. subtilis* by a procedure involving partitioning between phases of polyethylene glycol and dextran followed by agarose gel filtration. Two peaks of rifampicin-resistant RNA polymerase activity eluted from the column: one eluted at a position consistent with the size of the glycerol gradient-purified enzyme, and the other was much smaller and could indicate that other rifampicin-resistant RNA polymerases are associated with PBS2 infection but not detected by the purification procedure of Table 1. Alternatively, the rifampicin-resistant RNA polymerase may dissociate under certain conditions, to yield a subassembly that is catalytically active.

### Comparison of polymerases

Like the RNA polymerase of B. subtilis, the rifampicinresistant enzyme from PBS2-infected bacteria required all four ribonucleoside triphosphates, Mg2+ and a DNA template for optimal activity (Table 2). The rifampicin-resistant RNA polymerase actively copied PBS2 DNA and the synthetic template poly(dA-dT), but was relatively inactive with other DNA templates (Table 2). The ability to copy poly (dA-dT) indicated that the template specificity of the PBS2 RNA polymerase is not limited to uracil-containing DNAs. To investigate the template specificity of this enzyme further, PBS2 and B. subtilis RNA polymerases were assayed with PBS2 DNA and phage Φe DNA at various concentrations of enzyme and template. The rifampicin-resistant enzyme was more than ten times as active with PBS2 DNA than with  $\Phi$ e DNA, while the B. subtilis RNA polymerase was more than ten times as active with Φe DNA than with PBS2 DNA (Fig. 4). As an indication of the fidelity of transcription by the phage polymerase, RNA copied in vitro from PBS2 DNA by the phage enzyme hybridised with high efficiency (45%) to denatured PBS2 DNA but failed to anneal to denatured De DNA (data not shown).

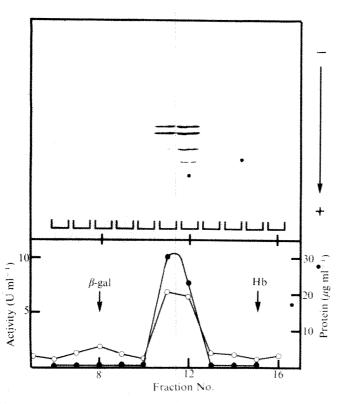


Fig. 2 Zone centrifugation of PBS2 RNA polymerase. Fractions 27 to 31 of the DEAE-Sephadex gradient (12 ml) were pooled and concentrated against Ficoll to a final volume of 2 ml. A sample of 0.5 ml of the concentrated fraction 5 enzyme (0.12 mg) was layered on to a 12 ml linear gradient of 10-30% (v/v) glycerol in buffer C containing 0.1 M KCl and 0.01 M MgCl and centrifuged at 2° C for 18 h at 39,000 r.p.m. in an International SB 283 rotor. Sedimentation standards,  $\beta$  galactosidase and haemoglobin, were sedimented in parallel in a duplicate gradient. Fractions of 0.75 ml were collected and 75  $\mu$ l was assayed as described in the legend to Table 1 with ³H-UTP (100 mCi mmoi ¹) (  $\blacksquare$  ), Each fraction was subjected to SDS polyacrylamide slab gel electrophoresis (8% w/v acrylamide)¹¹¹.  $\bigcirc$ , Protein concentration.

The PBS2 and B. subtilis RNA polymerases differ not only in template specificities but also in their sensitivities to rifampicin, streptolydigin and antiserum against B. subtilis RNA polymerase. The PBS2 enzyme was completely resistant to the drugs and largely resistant to the antiserum, while the B. subtilis enzyme was strongly inhibited by each agent (Table 3).

To determine whether the rifampicin-resistant RNA polymerase is present only after PBS2 infection, we tested for its presence in uninfected bacteria, in PBS2-infected bacteria and in bacteria infected with PBS2 after addition of rifampicin.

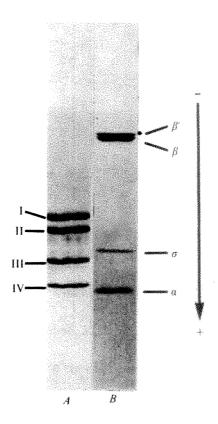


Fig. 3 SDS polyacrylamide slab gel electrophoresis of B. subtilis RNA polymerase and PBS2 RNA polymerase. PBS2 RNA polymerase (A) (3  $\mu$ g of fraction 6 enzyme) and B. subtilis holoenzyme (B) (a mixture of 4  $\mu$ g of fraction 5 holoenzyme and 0.5  $\mu$ g of fraction 7 sigma, prepared as described previously were subjected to SDS polyacrylamide slab gel electrophoresis (8 % w/v acrylamide)<sup>11</sup>

Rifampicin-resistant RNA polymerase activity was assayed at the DEAE-cellulose and phosphocellulose chromatography steps of the procedure of Table 1. Only the infected bacteria contained measurable rifampicin-resistant enzyme activity (Table 4). Thus, the PBS2 RNA polymerase is apparently not present in uninfected B. subtilis but appears after PBS2 infection even in the presence of high concentrations of rifampicin.

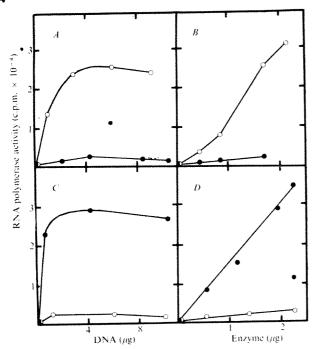
### Implications

The isolation of a new rifampicin-resistant RNA polymerase from PBS2-infected B. subtilis provides a likely explanation for

Table 2 Requirements for PBS2 RNA polymerase activity

	Activity	
	(%)	
Complete system	100	
-ATP	< 1	
CTP	1	
-GTP	9	
Mg <sup>2+</sup>	6	
$-Mg^{2+} + Mn^{2+}$	22	
+0.15 M KCl	13	
PBS2 DNA	<: 1	
+ poly(dA-dT)	70	
+Φe DNA	9	
+ <b>Φ29 DNA</b>	17	
+β22 DNA	4	
+Φ105 DNA	6	
+ B. subtilis DNA	16	

Assays were carried out as described in the legend for Table 1. The reaction mixture contained 0.9 µg fraction 5 PBS2 RNA polymerase. Except where noted, each assay contained 4 µg PBS2 DNA or 6 µg of the other templates. For the complete system, 100% activity was 17,000 c.p.m.



Template specificity of PBS2 RNA polymerase and B. subtilis RNA polymerase. B. subtilis holoenzyme (1.7 µg purified as described in the legend to Table 3) (A) and fraction 5 PBS2 RNA polymerase (1.9 µg) (C) were assayed as described in the legend to Table 3 with varying amounts of Φe DNA (○) and PBS2 DNA (♠). Various amounts of B, subtilis holoenzyme (B) and PBS2 RNA polymerase (D) were assayed with ♠ DNA (6 µg per assay)(○) and PRS2 DNA (4 µg per assay)(△) Φe DNA (6 μg per assay)(○) and PBS2 DNA (4 μg per assay)(●).

the drug resistance of phage growth during the later stages of the PBS2 lytic cycle. How is this new RNA polymerase initially produced in cells that have been inhibited with rifampicin-before infection? One possibility is that either the PBS2 RNA polymerase, a catalytically active subassembly of that enzyme, or another phage RNA polymerase is encapsulated in the PBS2 virion and injected along with the phage genome at infection. The injected RNA polymerase could then direct the synthesis of additional PBS2 RNA polymerase and other phage proteins independently of the host RNA polymerase. Other possibilities are that the phage injects a protein that confers drug resistance on the host RNA polymerase or that the phage genome is initially transcribed by an unidentified rifampicin-resistant

Table 3 Resistance of PBS2 RNA polymerase to inhibitors of B. subtilis RNA polymerase

Enzyme	Addition	Activity (%)
B. subtilis RNA polymerase	Rifampicin	100
PBS2 RNA polymerase	Streptolydigin Antiserum	8 100
	Rifampicin Streptolydigin Antiserum	100 103 68

B. subtilis RNA polymerase holoenzyme was purified by DEAE-Sephadex chromatography as described previously <sup>10</sup> but omitting DNA cellulose chromatography. B. subtilis RNA polymerase (3.4 µg per assay) and fraction 5 PBS2 RNA polymerase (1.9 µg per assay) were assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend to Table 1 with <sup>3</sup>H-UTP (100 mCl. assayed as described in the legend t assayed as described in the legislation and in the presence of 5 µg rifampicin, 30 µg streptolydigin or 200 µg anti-B, subtilis RNA polymerase antiserum<sup>12</sup>. The template for B. subtilis RNA polymerase was Φe DNA (6 µg) and the template for PBS2 RNA polymerase was PBS2 DNA (4 µg). One hundred per cent activity for *B. subtilis* RNA polymerase and PBS2 RNA polymerase was 58,500 c.p.m. and 35,300 c.p.m. respectively.

RNA polymerase of the host. A recent report indicated that transcription by the phage N4 immediately after infection of E. coli is also resistant to rifampicin<sup>13</sup>. It will be interesting to compare the mechanisms of drug resistant transcription by these unrelated phage.

The purified PBS2 RNA polymerase is complex in structure and, like the RNA polymerase of E. coli and B. subtilis, is apparently composed of multiple subunits. Bacterial RNA polymerases are known to interact with phage-induced polypeptides including the protein products of known regulatory genes<sup>14-18</sup>. The PBS2 genome is unusually large (1.9 imes 10<sup>8</sup> daltons)19 and is undoubtedly transcribed in a defined temporal sequence although the programme of phage transcription has not been investigated. It is tempting to speculate that the PBS2 RNA polymerase, like the multi-subunit RNA polymerases of bacteria, interacts with other phage proteins that regulate the expression of different classes of phage genes during the PBS2 lytic cycle.

Table 4 Appearance of rifampicin-resistant RNA polymerase after infection by PBS2

Extracted from Uninfected B. subtilis	DEAE-cellulose enzyme activity (U mg <sup>-1</sup> ) <0.01	Phosphocellulose enzyme activity (U mg <sup>-1</sup> ) <0.15
PBS2 infected B. subtilis	0.33	22.7
B. subtilis infected by PBS2 in the presence of rifampicin	0.27	18.3

Three cultures of B. subtilis 3610 were grown in medium 121A at 1 nree cultures of B. subtuts 5010 were grown in mediatin 1213 at 32° C to a density of  $2.5 \times 10^8$  cells per ml. One culture (25 l) was infected by PBS2 at a multiplicity of three. A second culture (12 l) was infected similarly by PBS2 5 min after addition of rifampicin (50 µg ml<sup>-1</sup>). Both cultures were collected 45 min after infection. A third uninfected culture (121) was collected at a cell density of 3.5 × 10s per ml. The purification procedure of Table 1 was followed until the phosphocellulose chromatography step. PBS2 RNA polymerase activity was measured as in the legend to Table 1.

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# letters to nature

# Enhanced low-energy gamma rays at balloon altitude in the Brazilian magnetic anomaly

The region of the Brazilian geomagnetic anomaly presents an interesting opportunity for the study of precipitation of charged particles of the inner radiation belt into the atmosphere below. Some recent studies based on direct and indirect observations (refs 1–3 and our unpublished work) show that the precipitating flux increases appreciably during the period of a geomagnetic storm. The present note provides another evidence in this direction.

As a part of the continuing cosmic ray programme at INPE, balloons with  $\gamma$ -ray and charged particle detectors were launched on October 7 and 20, 1973. They reached the ceiling altitude of  $\approx 3.5$  mbar and remained there for few hours measuring  $\gamma$ -ray and charged particle fluxes.

The magnetograms obtained at São José dos Campos indicate that on October 20 the balloon was launched during a magnetically disturbed period. It seems that a sudden commencement of small amplitude at about  $0645 \, \text{UT}$  on October 16, 1973, preceded the start of a disturbance in the Earth's magnetic field. It seems that more than one disturbance occurred, so that the period of the balloon flight is a mixture of the main phase and the recovery phase of a magnetic storm. This disturbance continued practically for the whole week. The purpose of this note is to discuss the change in the  $\gamma$ -ray and charged particle fluxes during the disturbed period in relation to those observed during the quiet period in conjunction with relevant geomagnetic and ionosphere observations.

The balloon launchings on both occasions were conducted at São José dos Campos (23°14′S, 45°51′W). On October 7, 1973, the balloon was launched at 0830 UT and it reached a ceiling height of 3.8 mbar but data were obtained for about 30 min only. Even with this short period of observation it is possible to get an idea of the 'normal' level of the γ-ray and charged particle fluxes at the ceiling height. On October 20 the balloon was launched at 0715 UT, attained height of 3.5 mbar at about 0915 UT and telemetered the data obtained for several

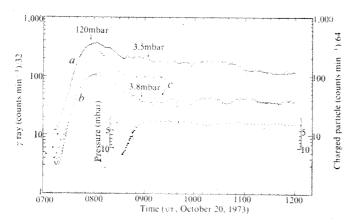


Fig. 1 Count rate as function of time for γ rays (a) and charged particles (b) for the October 20, 1973 flight. Also shown for comparison are the corresponding quantities for the October 7, 1973 flight (c), time shifted so as to coincide with the October 20 curves during the flight ascent. The curve displaying the barometric pressure variation during the October 20, flight is also indicated.

hours. Since the ceiling height of both flights is approximately the same it is meaningful to compare the results and look for effects due to the geomagnetic disturbance.

The detector in both flights consisted of a 4"×4" NaI (T1) scintillator surrounded by a plastic scintillator 1 cm thick. The latter operated in anticoincidence for separating out charged particles. The energy threshold of the detector was such that the minimum energy at the top of the atmosphere of charged particles capable of triggering the detector was 7.0 MeV for electron and 30 MeV for protons. The equivalent area of the NaI (T1) crystal detector is 121.62 cm². The details regarding the calibration and other relevant information can be found in ref. 4.

The curves of the integral count rates as function of time for  $\gamma$  rays and charged particles measured on October 20, 1973 are shown in Fig. 1. The  $\gamma$ -ray count rate is integrated from 0.9 to 18 MeV. A curve for the pressure measurements is also included in Fig. 1. For comparison the curves for  $\gamma$ -ray and charged particle fluxes in the October 7 flight are also shown in Fig. 1, time shifted so as to superimpose the two curves during ascent, without any change in the vertical scale. Starting from the level of maximum count rate at 120 mbar there is a substantial increase in the  $\gamma$ -ray flux. The charged particle flux also shows noticeable enhancement although not as large as in the case of  $\gamma$  rays. At the ceiling height the charged particle seems to have reached almost the normal equilibrium level.

The increased count rate on October 20 can be attributed to the high level of geomagnetic activity prevailing at that time. (The omnidirectional character of the detector and the short time interval between flights exclude the possibility of interpreting the higher  $\gamma$ -ray count rate on October 20 as being due to contribution from any known or unknown celestial source.)

The fact that the increase in  $\gamma$ -ray flux is considerably larger than the charged particle flux may be attributed to the fact, that the  $\gamma$ -ray flux observed is primarily due to bremsstrahlung produced by the downward moving electron flux. Whereas the charged particle detector will register only the flux reaching the ceiling level, the  $\gamma$  ray counts are due to the integrated radiation produced in the layers above the ceiling height. Since the charged particle flux undergoes a decrease with depth, it would be reasonable to expect that the increase in the low energy  $\gamma$ -ray flux should be larger than the directly measured charged particle flux.

To verify this we have calculated from the measured energy loss spectrum in the crystal, the energy spectrum of the incident  $\gamma$ -ray flux (Fig. 2a). For the period 1100-1230 UT (Fig. 2a) the γ-ray count rate was at a lower level, where it stayed for the remainder of the flight. We therefore assume that this period is representative of normal conditions, when no enhanced particle precipitation is taking place. By contrast, the interval 0900-1030 UT (Fig. 2b) covers the period during which the maximum enhanced precipitation is seen to occur. The spectrum for the period 1100-1230 UT is a power law  $\approx E^{-1.1}$  for the entire energy range. For the period 0900-1030 ur the power law is  $\approx E^{-2.2}$  in the range 0.9-2.0 MeV and  $\approx E^{-1.1}$  in the range 7.0-18.0 MeV. In the range 2.0-7.0 MeV, the spectrum is not well represented by a power low in energy. Thus, there seems to be a relatively greater increase in the low energy flux than in the high energy region. Assuming that the increase in the low energy \gamma-ray flux is due to bremsstrahlung from electrons with an energy spectrum given by Pfitzer et al. for

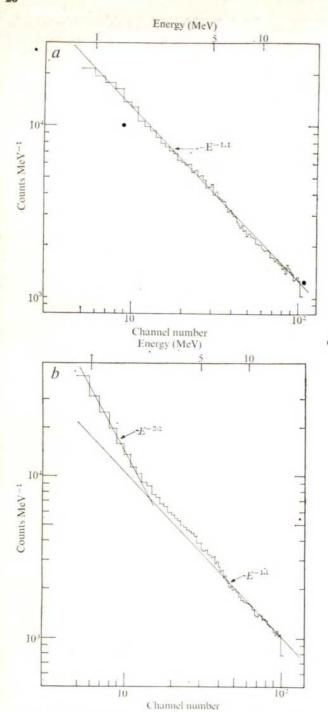


Fig. 2 a, Energy spectrum of the incident γ-ray flux during the period 1100–1230 UT on October 20, 1973. b, Energy spectrum of the incident γ-ray flux during the period 0900–1030 UT on October 20, 1973. 0.164 MeV per channel.

L=1.3, we estimate that these y rays are originating mainly from electrons with energies up to 10 MeV.

Increase in the precipitating particle flux at much lower energies (>40 keV) has also been detected during the period in question by means of change in ionospheric conditions above São José dos Campos. Figure 3a and b show the ionograms taken at 9030 and 1000 UT on October 20 at Cachoeira Paulista located near São José dos Campos. Development of several well stratified ionospheric layers between 100 and 200 km is visible in the ionogram taken at 1000 ur. For comparison we have presented ionograms taken at the same hours on October 13 before the storm (Fig. 3c, d) and on October 26 after the storm (Fig. 3e, f).

Ionograms for October 7, corresponding to the first balloon flight, were not available. The presence of the sporadic E layer at 1000 UT on October 26 introduces some difficulty in interpreting the echoes from the 100-200 km region. But, the nature of the traces obtained at these hours on October 13, and at 0930h on October 26, does suggest that the well defined layer between 100 and 200 km seen on October 20 results from the particle precipitation during the magnetic disturbance on this day.

The association between the increased precipitating flux of high energy particles and the geomagnetic activity has already been noted2. The exact manner in which the geomagnetic field variations produce the increase electron precipitation is not clear. But short-period fluctuations (of the order of few minutes) in the geomagnetic field are believed to provide an effective mechanism (J. M. da C., N. O. T., and D. B. R., unpublished). Records of the total geomagnetic field intensity obtained at São José dos Campos shows that such short period fluctuations are present, and they could thus contribute significantly to the increased precipitation of electrons. An interesting feature

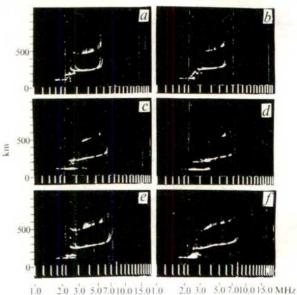


Fig. 3 The ionograms taken over Cachoeira Paulista on October 20. a, at 0930 UT; b, at 1000 UT. Also shown for comparison are the ionograms taken at the same hours on October 13, (c and d) and October 26 (e and f), which may be considered to be normal days. The frequency sweep in the ionograms is from 1 MHz to 20 MHz and the height markings are at 100 km intervals

Frequency

pertaining to the October 7 charged particle measurements is the apparent periodicity of about 30 min seen in the records at the ceiling altitude. There is also some suggestion of a periodicity in the curve of charged particle count rate for the flight of October 20. The corresponding curve for γ-ray flux also shows fluctuations but the periods seem to be shorter. The possible existence of a similar 30 min periodicity has been noted previously1,2 and related to the configuration of the magnetosphere over the anomaly region or to radial diffusion. More observations are needed to establish on more firm grounds the existence of such periodicities before we could attempt any detailed explanation

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### Observations of the Orion nebula at 100µ m

WE observed the Orion nebula at coarse spectral resolution during flights on March 13 and 14, 1974, using a cooled grating spectrometer carried on NASA's Lear jet.

The instrument covered the spectral range from 80 to 125 µm at a resolution of 8 µm. It used a Ga: Ge photoconductive detector with an instrinsic long wavelength cutoff at approximately 130 µm, and radiation shortward of 70 µm was blocked by black polyethylene and a Yoshinaga filter. The field of view was roughly 5' square, which is large enough to include most of the brighter portions of the Orion 100 µm source, according to D. A. Harper (unpublished).

To obtain a spectrum the grating was rotated to throw light of different wavelengths on to the detector. At each grating position measurements were made on and off the source, and the difference taken to be the source signal level. Several runs through the spectral bandpass were completed, taking steps of about 5  $\mu$ m, and a raw spectrum was obtained by averaging the values for a given grating position weighted according to the reciprocal of their variances.

Calibrations took two forms. The spectral calibration came from observations of the Moon which had to be taken on separate flights, since the elevation angles accessible to the Lear telescope are restricted and useful observing time is limited to about one hour per flight. But lunar spectra obtained on the mornings of March 11, 12 and 13 and the evening of March 7 are nearly identical. These spectra were used to correct for instrumental profile and atmospheric water vapour absorption, and for this purpose the moon was assumed to radiate as a 390 K blackbody<sup>1</sup>.

The intensity calibration is based on a temperature for Venus (289 ± 20 K) from Armstrong et al.2, and utilises Venus observations from March 11, 12 and 13.

Our Orion nebula spectrum corrected for instrument profile and atmospheric water vapour absorption is shown in Fig. 1 (left hand). Figure 1 (right hand) shows the lunar

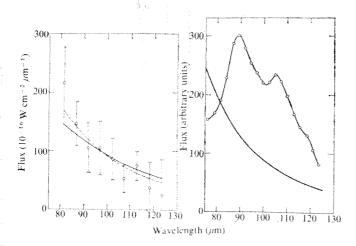


Fig. 1 Right-hand side, Orion nebula spectrum at 8 μm resolution. The blackbody curves are diluted by factors of 83 for 100 K (---) and 2 for 60 K (---). Error bars are one standard deviation. Left-hand side, Calibration spectrum of the Moon ( −○), with superposed 390 K blackbody (—). Errors are too small to plot (<2%).

spectrum obtained on the morning of March 13. The water vapour absorption feature at 100 μm is relatively weak, and this is expected to be the case for all our observations, since they were made from several kilometres above the tropiopause.

Superposed on our Orion data are 60 and 100 K blackbody curves diluted to give the right intensity, since the source does not fill our beam and may not be optically thin. Both curves fit the data, although grain emissivities of the form  $\lambda^{-n}$  give a better fit. Thus the best fits at 60 and 100 K are given by n=1.7 and 0.9 respectively, but in view of the low signal-to-noise ratio the difference from a blackbody is not statistically significant. Pure blackbody curves for temperatures below 40 K do not fit the data well, and the fit improves steadily for higher temperatures. Although our spectrum is somewhat steeper, our results are in good agreement with those of Erickson et al.3. Our flux calibrations also agree with the photometric data of Low and Aumann's and Hoffmann, Frederick and Emery'. The error bars in Fig. •I represent our standard deviation, and are derived from the standard deviations of the individual readings.

Our main conclusion is that our results are consistant with a thermal emission spectrum produced by cool interstellar grains radiating at a wavelength much longer than the grain size. If atomic or molecular lines dominate the 100 µm flux, our spectral resolution is too coarse to detect them, but in any case there would have to be some conspiracy of lines to produce the apparently rather smooth spectrum we detect.

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### **Bathonian volcanicity** and North Sea rifting

SEVERAL years ago<sup>1,2</sup> we proposed that the Bathonian and Aptian fuller's earths of southern England, which are montmorillonitic clays, are true bentonites and, therefore, of volcanic origin (see also ref. 3). With the evidence of Bathonian volcanicity associated with rifting in the North Sea, we can now develop the story a stage further.

Two new publications<sup>4,5</sup> outline the pre-Tertiary structure of the North Sea region in terms of a series of troughs separated by upstanding massifs or 'platforms'; we here adopt the more detailed reconstruction of Naylor et al.5 (Fig. 1). They have proposed a model in which thermal expansion of the crust and topmost mantle, perhaps consequent on the rise of a plume, leads to updoming and tensional collapse in the central region. This gives rise to trilete junctions at which three potential spreading ridges diverge. The Forties, Ekofisk and Northern North Sea (or Viking4) troughs form such a rift-rift-rift

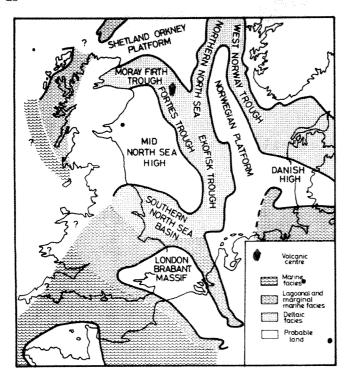


Fig. 1 Structural and palaeogeographic setting of Britain and adjacent areas during the Bathonian.

junction, and it seems to be highly significant that Bathonian volcanicity occurs at least approximately, at this junction4. The implied initiation of a major phase of updoming and rifting at this time can be supported on palaeogeographic grounds.

Considering the Jurassic geology of north-western Europe as a whole, it can be said that the gradual trend, in the early part of the Period, towards increasing marine conditions, with shallow seas progressively deepening and transgressing over the land, was reversed in the Middle Jurassic. All the way across from Britain to northern Germany the sedimentary facies tend to become more regressive from the Toarcian to the Bathonian, before more marine conditions became re-established in the Callovian. The sequence in Yorkshire, for instance, illustrates this well. Marine Lower Toarcian clays and shales pass up into shallower marine sands and ironstones in the Upper Toarcian and Aalenian, and then to mixed, shallow marine, and non-marine deltaic deposits in the Bajocian. The Upper Bajocian and Bathonian are wholly non-marine deltaic, and are replaced once more by marine deposits from the Lower Callovian upwards.

Viewed regionally, the area of greatest regressive tendency within the depositional regime can be identified as the central and northern North Sea (Fig. 1). In Yorkshire, marine influence during the Bajocian diminished northwards<sup>6</sup>, as it did in north-western Germany and southern Scandinavia7. In Andøye, northern Norway, the Middle Jurassic is wholly non-marine and deltaics. The Bathonian of north-eastern Scotland is developed in deltaic facies, with no hint of marine deposits, whereas in western Scotland it is partly deltaic but mainly brackish-lagoonal and marginal marine. Across England and northern France, increasingly marine conditions can be inferred towards the south, and there is an increase in the proportion of calcareous deposits, signifying minimal influx of terrigenous sediment. In the Bathonian, for instance, the deposits are entirely deltaic in Yorkshire, mixed marginal marine and deltaic in the eastern Midlands, marginal marine in the southern Midlands and the Boulonnais and fully marine in Normandy and Dorset. A major source of coarse clastic sediments must be postulated in the North Sea region. Petrological evidence6 indicates this to be, in all probability, reworked late Palaeozoic sandstones, huge volumes of which were uplifted on the Mid-North Sea High and adjacent positive areas. (The London-Brabant Massif does not seem to have been a major sediment source.) The finer sediments were probably derived partly from uplifted Triassic and Liassic rocks in the same areas

Returning to the Bathonian fuller's earth of Somerset, the thickness of 1.5 m for a pure or almost pure montmorillonitic clay seems to be excessive for a single bentonite formed from ast carried some 450 miles from the volcanic centre. The most likely explanation is that a series of major eruptions took place during a restricted part of the Bathonian, at a time datable as Retrocostatum Zone, when normal terrigenous illite-kaolinite clay sedimentation was much reduced in the Bath area. A certain amount of reworking and ponding of ash deposits in smal depressions would have enhanced the thickness locally. Montmorillonite is generally rare in British Jurassic clays and has no been found in beds older than Bathonian. We predict that bentonitic or montmorillonite-rich clays may be found over a wide region in late Bathonian deposits of central and northerr England and Scotland; and they may provide a good stratigraphical marker horizon. On the basis of the bentonite mineralogy and chemistry, and the types of igneous rocks to be expected in early phases of continental rifting, the further prediction can be made that the lavas and/or tuffs will be basic to intermediate and alkaline in composition.

The initial rifting in the North Sea was not followed by seafloor spreading, and therefore, the British Isles did not separate completely from the continent of Europe; only further trough subsidence accompanied by marginal faulting occurred, con stituting what has been termed a 'Failed Arms System5'. This i in contrast to the subsequent Cretaceous rifting to the west and south-west of the British Isles, which also seems to have been associated both with volcanicity, judging from the Aptiar bentonites and other evidence, and upwarping of the continen tal crust, as marked by regional patterns of overstep and sedimentation10.

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### Evidence of precursive compression for two deep earthquakes

Dziewonski and Gilbert have claimed that they car detect changes at a seismic source, starting 80 s before the origin time inferred from (impulsive) body wave arriva times. I believe, however, that their conclusion is almos certainly an artefact of their data analysis technique.

Dziewonski and Gilbert used the 'stacking and stripping procedure4-6 to construct Fourier transforms of tim

derivatives of the source time functions. That technique is applicable only at very long periods; as periods become shorter, lateral variations in dispersion become important. Dziewonski and Gilbert computed the transforms of the source rate functions only for periods of more than 78 s. Their procedure is exactly equivalent (if their reconstruction of the source rate transforms is correct) to taking the transforms of the source rate functions over the entire frequency range and then filtering the transforms by setting to zero all Fourier components with periods of less than 78 s, without changing the longer periods.

That type of filtering is 'non-causal.' If a delta function at t=0 is passed through this filter, energy will emerge from the filter at negative times, before the source energy arrives (Fig. 1). Dziewonski and Gilbert's procedure is de-

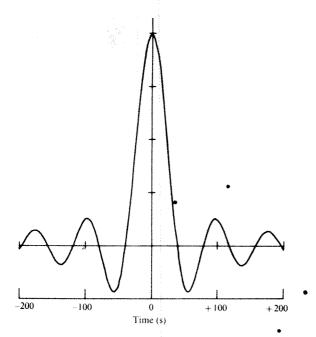


Fig. 1 Impulse response of the filtering procedure used by Dziewonski and Gilbert.

fensible only in cases where almost all of the source rate energy is concentrated in the ultra-low frequency band. But more than 85% of the energy of the (far field) source functions is contained, however, in the short periods, and so they cannot determine an accurate origin time from the free oscillation data set. If the Fourier transforms which they inverted to get their Fig. 2 had included all periods (instead of only the ultra long periods) it is quite likely that the time functions would all have been uniformly zero before the origin time.

The estimate that more than 85% of the spectral energy density was outside the frequency range considered by Dziewonski and Gilbert was derived by combining Aki's statistical scaling rule² with observational data. Aki's rule states that the far field displacement (or source moment rate) spectrum will be flat up to some 'corner frequency', above which amplitude falls proportionally to ω⁻². Because the energy density of the source moment rate is constant below the corner frequency, the total energy in a frequency band from zero to a cutoff below the corner frequency is proportional to ω. The results of Wyss and Molnar³ indicate that 0.1 Hz is a conservative estimate of corner frequency for the deep earthquakes considered by Dziewonski and Gilbert. Thus their cutoff frequency (0.013 Hz) eliminated well over 85% of the signal energy.

The conclusion that the hypocentral region began to undergo compresion 80 s before the observed origin time is, therefore, unjustified.

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DRS DZIEWONSKI AND GILBERT REPLY: Although Geller discusses the problem of the rate of release of seismic energy, his criticism of our paper is concerned with our statement that the isotropic part of the moment rate tensor is precursive by 80 s. In this reply, we address ourselves to this precursive behaviour.

If  $g(\omega)$  is the Fourier transform of f(t), then  $g(-\omega)$  is the Fourier transform of f(-t). If  $g(\omega) = g(-\omega)$  then f(t) =f(-t). If  $g(\omega)$  is an even function of frequency then f(t)is an even function of time. If f(t) is an even function of time it is precursive. The experimental evidence for the isotropic part of the moment rate tensor shows its cosine transform, an even function, to be large at low frequencies, and its sine transform, an odd function, to be small everywhere. Consequently, the isotropic part of the moment rate tensor has a Fourier transform that is an even function of frequency, from which follows that it is an even function of time, and, necessarily, precursive. The spectrum of the isotropic part of the moment rate tensor is very small for frequencies above 0.0048 Hz (0.03 s<sup>-1</sup>). The precursor therefore should persist into negative time for about 105 s  $(\pi/0.03 \text{ s}^{-1})$ , which is in accord with our original, conservative estimate of 80 s.

Mr Geller shows a symmetric function of time that has a nearly flat cosine spectrum below 0.01313 Hz (0.0825 s<sup>-1</sup>). It is nothing more than a low-passed delta function. The observed spectrum of the isotropic part of the moment rate tensor decays rapidly with increasing frequency, and is nearly zero above 0.0048 Hz (0.03 s<sup>-1</sup>). Consequently it is not a low-passed delta function and must therefore be precursive.

The only way to invalidate this conclusion is to have the sine transform differ significantly from zero at very low frequencies, below 0.002 Hz (0.0125 s<sup>-1</sup>).

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# Correlation of strain and velocity during dilatancy

PRESENT earthquake mechanism models are predicated on the hypothesis of the formation of a dilatant zone in the region of the proposed break and the time history of physical rock properties that are a function of crack porosity. For example, field observations have shown drops in compressional wave velocity in earthquake areas prior to events. The anomaly persists for a time dependent on the source dimensions of the impending shock, and returns to normal prior to the event1-4. Velocity measurements made during laboratory fracture tests have shown a decrease in compressional wave velocity above approximately 80% of the fracture stress<sup>5,6</sup>. The effect is most pronounced normal to the direction of greatest compression. Similarly, as the fracture stress is approached the inelastic volumetric strain is the greatest7, indicating a definite correlation between velocity and inelastic volumetric strain.

Here we correlate spatial variations in compressional velocity with spatial variations in strain over the extended time interval required for the rock to fail under constant stress. A right circular cylinder of Westerly granite was statically loaded in uniaxial compression and velocity observed between the transducer pairs shown in Fig. 1. Coincident illumination and viewing holographic interferometry (refs 8, 9 and N. D. Meyer and H. S., unpublished) enabled us to observe continuously and interpret the entire strain field on the rock specimen.

At the start of the experiment a stress of  $2.28 \times 10^7$  kg m<sup>-2</sup> (2,240 bar) was applied for 24 h; then the load was incremented to be a constant  $2.50 \times 10^7$  kg m<sup>-2</sup> (2,450 bar) and held until failure. Strain (for the last 12 h only) is given in Fig. 1 as radial strain parallel to each of the velocity paths.

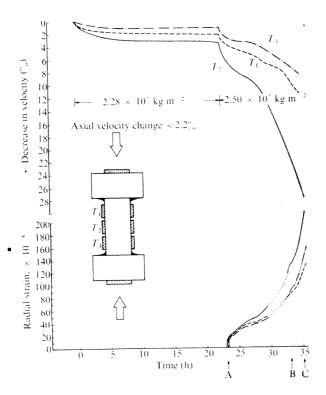


Fig. 1 Decrease in velocity (%) as a function of time over three paths,  $T_1$ ,  $T_2$  and  $T_3$  (see insert), for two creep episodes. The sample failed after 12 h at  $2.50 \times 10^7$  kg m<sup>-2</sup> (2,450 bar). The average radial strain over the same three paths for the second creep event is shown at the bottom of the diagram. The radial strain was calculated from 15 holograms which covered the entire last creep event. The first exposure of each hologram was taken immediately after the second exposure of the preceding one. This yields a complete strain history of the sample.

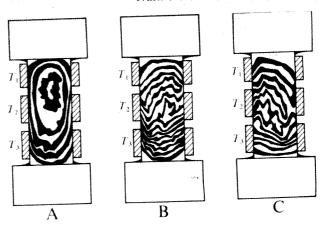


Fig. 2 Tracings of double exposure holograms at times A, B, and C as indicated in Fig. 1. Hologram A shows the effect of an axial stress increase from  $2.28 \times 10^7$  kg m<sup>-2</sup> (2,240 bar) to  $2.50 \times 10^7$  kg m<sup>-2</sup> (2,450 bar), B and C show the effects of creep during 44 min and 13 min intervals con mencing 2.25 h and 0.25 h before failure, respectively.

The total axial strain, measured with a displacement transducer, was nearly linear over the last 12 h and amounted to  $10^{-3}$ . This means that the sample was dilatant during the last episode and the inelastic volumetric strain curve will have the same general shape and characteristics as the radial strain depicted in Fig. 1.

Figure 2 shows three tracings of selected holograms, double exposed at times A, B, and C as shown in Fig. 1. Each hologram contains fringes which can be thought of as 0.3  $\mu$ m contour lines in a topographic map of the surface change of the cylinder. The fringes result from the interference of two slightly different images projected into space.

Hologram A shows the change in surface displacement due to increasing the stress to  $2.50 \times 10^7$  kg m<sup>-2</sup> (2,450 bar). The contours are smooth and form easily identifiable ellipses. The strain is concentrated at the top of the sample and may be characterised as a broad asymmetric bulge along the axis of the sample. The strain field in hologram B taken eight hours after A is much more complex. The fringes are irregular, the strain concentration has shifted toward the bottom of the sample, and the strain ellipse strikes NE. The distortion of fringes is attributed to surface crazing prior to spallation. The contour fringes are much more closely spaced than in A, indicating a larger strain accumulation. The interval between exposures was 44 min. Hologram C was completed 15 min prior to failure; the interval between exposures was 13 min. The greatest strain is still concentrated in the lower half of the sample, and the fringes are distorted, but the strain ellipse now strikes NW. This suggests that the strain field in the sample is not only inhomogeneous but regions of peak strain migrate through the sample. From an examination of all 15 holograms, three such changes in orientation were noted. It seems that two incipient conjugate faults form prior to failure with the strain accumulating on each independently.

The transverse velocity decreased rapidly following the stress increase at approximately 24 h. The slight change in slope at 28 h was then followed by a precipitous plunge toward failure. The radial strain data in Fig. 1 were obtained from holograms along three lines corresponding to the transverse velocity paths. The similarities between the velocity and strain histories are apparent. First, the location of greatest velocity falloff corresponds to the region of largest inelastic strain. Second, there is a definite correlation in the shape of both sets of curves. The transients in all six curves persist for approximately the same period, and the accelerated segments, prior to failure (tertiary creep), all commence at the same time.

So there is a strong correlation between the local inelastic strain and the velocity. The large variations in velocity over

several centimetres distance were unexpected. In addition, the resolution of the holograms presented here shows holographic interferometry contributes significantly in unravelling the local changes in inelastic strain that precede failure.

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### Tholeiite, alkali basalt and ascent velocity

ALKALI basalts occur in a great variety of geological environments. It would, therefore, be surprising if only one mechanism were responsible for all occurrences, but we attempt here to explain some features of alkali basalt volcanism which are not readily understood in terms of the deep origin theory.

Engel et al.1 concluded that the great majority of rocks from the ocean floor were tholeiitic basalts, and that the occurrence of oceanic alkali basalts was restricted to the tops of volcanic islands and sea mounts This generalisation is still largely valid, though, apparently, a greater variety of rocks types are found in the ocean basins2,3 than was admitted by Engel et al.1.

The pillow basalts at the top of layer 2 of the oceanic crust are believed to comprise mainly tholeiite, produced at active spreading ridges. The most important parts of many oceanic islands also seem to be tholeiitic; this has been observed for Hawaii<sup>2</sup>, Reunion<sup>4</sup>, and several other islands. Plateau basalts such as the Columbia River Basalts and the Deccan Basalts, are predominantly tholeiitic, as are gabbroic layered intrusions such as Skaergaard and Muskox.

Alkali basalts are frequently produced during the last stage of oceanic volcanism as has been described for Hawaii and Reunion Island, but there are some oceanic volcanic islands, such as the Canary Islands, which seems to be made up mostly of alkali basalts. Alkali basalts also occur at the ocean floor, near ridge axes3 and transform faults7. The Red Sea8,9 and the Afar region10, seem to be underlain by alkali basalt or tholeiite with definite alkaline characteristics. Alkali basalts are typical of the African rift

Alkali basalts are frequently associated with the tholeiitic flood basalts as is the case in Britain and Greenland11. Marginal oceanic basins seem to contain tholeiites which tend to have alkaline characteristics12.

Laboratory experiments<sup>13,17</sup> on the origin of alkali basalts and tholeiites, have been interpreted to indicate that tholeiitic basalts have been formed by a relatively large degree (25%) of partial fusion of a peridotitic parent material at a shallow depth in the upper mantle; and alkali basalts are thought to come from a great depth in the upper mantle. Geochemical arguments based on the contents of trace elements in alkali basalts and their presumed peridotitic parent material, have led to the conclusion that alkali basalts can only be produced by small degrees of partial fusion 18.

We propose an alternative model. Because of the poor thermal conductivity of silicates, vertical movements in the mantle have been considered to be, in general, adiabatic. Thus, the rate at which the temperature changes in a mantle diapir is, to a first approximation, given by the adiabatic gradient: 0.3°-0.5° C km<sup>-1</sup>. If the diapir rises to the surface and is already at a temperature close to its solidus temperature, partial melting can be anticipated, because the solidus temperature of potential upper mantle materials varies with depth by about 4° C km<sup>-1</sup> (ref. 14). If, however, the temperature contrast between the diapir material and the surrounding upper mantle becomes large, and/or when the apward vertical velocity of the moving diapir becomes small, the diapir may cool faster than adiabatic cooling. because heat transport by conduction may then become significant as compared with heat transport by mass transfer.

Numerical calculations 19,20 indicate that at depths of more than 25 km, and velocities of less than 1 cm yr<sup>-1</sup>, the rate of cooling of a massive, upward moving current of silicate crystal mush, becomes greater than the variation of the basalt solidus temperature with depth. When conductive cooling becomes important relative to adiabatic cooling in rising mantle diapirs, the amount of partial fusion of the diapir will diminish; thus, the liquid produced because of . decompression in the diapir will tend to be more alkaline than would be the case for a rapidly rising diapir, in which conductive cooling would be negligible.

In the case of Hawaii, there is evidence that tholeilte is generated at a depth below 60 km (ref. 21) by about 30% partial infusion of a herzolitic upper mantle. Seismic evidence<sup>21</sup> suggests upward lava velocities considerably in excess of 1 cm yr<sup>-1</sup>. If, however, the lava moved upward more slowly, as may occur during the waning stage of volcanic activity, then it may start to precipitate before reaching the surface and will then tend to develop alkaline characteristics which will be further accentuated by contamination with wall rock. Contamination is more serious in slowly moving lavas.

The theory of deep origin for alkali basalts, as applied to Hawaii, suggests that alkali basalt also originates at depth in excess of 60 km, below the oceanic lithosphere, in the asthenosphere or even deeper. The prolonged alkali basaltic activity of islands in the Atlantic, such as the Western Canary Islands, is therefore difficult to understand. Likewise, the apparent total absence of tholeiites on several islands in the Atlantic is not readily explained by the theory of deep origin for alkaline basalts.

Another situation in which the rate of ascent may determine the type of basalt, is the case of very slow seafloor spreading. Basalt erupted at an active spreading centre is thought to be generated by partial melting, as a result of pressure decrease, in upward moving mantle material coming from the low velocity zone 19,20,22. Low spreading velocities imply low rates of ascent underneath the ridge centre. This situation has been modelled quantitatively, and it was found that for vertical velocities of 1-8 cm yr<sup>-1</sup> the composition of ridge basalt does not depend on the rate of ascent But for vertical velocities of less than 1 cm yr<sup>-1</sup> the basalt composition became more alkali rich than tholeiite.

Thus, it can be understood why, in regions characterised by very low plate velocities, alkali basalts or tholeiltes with definite alkaline tendencies are frequently found. An example is the Red Sea8 which has a plate velocity of less than 1 cm yr<sup>-1</sup>. There are alkali basalts at the Goringe Bank in the Atlantic23. This location is situated in the magnetic

quiet zone which itself possibly results from a very slow rate of seafloor spreading24.

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### Ubiquitous trisulphur radical ion S<sub>3</sub>

THE colour of blue ultramarine and the deep blue colour of alkali polysulphides in electron pair donor solvents have been attributed to the disulphur radical ion, S<sub>2</sub><sup>-</sup> (ref. 1). That assignment is erroneous and I here establish the correct identity of the species, emphasise the variety of situations in which it is encountered and point out its possible role as an intermediate, in transformations involving elemental sulphur, or sulphide ions.

A blue colour develops when sulphur is heated with water and traces of some basic salt2.3. Blue solutions are also formed by sulphur in alkali halide melts4, in liquid potassium thiocyanate above 300° C (refs 5 and 6), by alkali polysulphides in basic solvents<sup>7-12</sup>, by electrochemical reduction of S<sub>8</sub> in dimethyl sulphoxide13-15 or LiCl-KCl eutectics16,17, and in the electrochemical oxidation of alkali metal polysulphides in eutectics18. The blue species is also present (as a minor component) in

dilute solutions of sulphur in ethylenediamine 19-21 and in the mineral lapis lazuli22,23 and it is formed from proteins containing fully combined cystine, for example, insulin in liquid ammonia<sup>24</sup>, from dimethylamine and elemental sulphur in hexamethylphosphoramide11, and in acetone solutions of sulphur containing potassium hydroxide25

The blue species is characterised by a visible absorption band at 610-620 nm, and it can be attributed to the trisulphur radical anion,  $S_3$  (refs 10, 12, and 15). It is well established that  $S_3$  (in the solid state) has an optical absorption at about 610 nm, which is associated with a Raman band at 534 cm<sup>-1</sup>, whereas S<sub>2</sub><sup>-1</sup> shows a Raman band at 594 cm<sup>-1</sup> associated with an optical absorption band at about 400 nm (refs 26-28). Furthermore, S<sub>a</sub>: exhibits a characteristic infrared band at 580 cm<sup>-1</sup> (refs 10, 11, and 26), (antisymmetric stretching mode), whereas S<sub>2</sub>. is, as expected, infrared inactive. The electron spin resonance (e.s.r.) spectra of  $S_3^{-}$  (ref. 29) and  $S_2^{-}$  (ref. 30) are also well characterised, and the technique has been used to detect S<sub>3</sub>. on the surface of the catalyst when elemental sulphur reacts with partially hydroxylated magnesium oxide at 400° C (ref. 31).

Although the existence of catenated species is a general feature of sulphur chemistry, and probably results from the relatively high  $\sigma$ -bond energy, compared with  $\pi$ -bond energy, for sulphur<sup>20</sup>, the formation of  $S_3^{-}$  in preference to other sulphur radical ions is not readily explained. Like other 19e triatomic molecules for example,  $Q_3^{-1}$  and  $ClO_2$  (refs 32, 33)  $S_3^{-1}$ will have C<sub>2v</sub> symmetry with SSS < 120°. The odd electron will occupy a molecular orbital which is S-S antibonding, but bonding with respect to terminal sulphur atoms<sup>34</sup>. The possibility of S · · · S interactions between terminal sulphur atoms must, therefore, be considered. For the  $S_3^{2-}$  ion, of known structure<sup>1</sup>, the separation would be 3.37 Å. According to the Walsh rules for triatomic molecules<sup>34</sup>, the SSS angle would increase slightly for  $S_3^-$  whereas the S-S distance should decrease compared with  $S_3^{2-}$ , so that the  $S \cdots S$  separation in  $S_3$  can be expected to be similar to that in  $S_3^2$ . Although this value is lower than the sum of the Van der Waal's ratio for sulphur (3.70 Å), it is considerably larger than the S-S intraring interactions in  $S_8^{2+}$  [ $d(S_3-S_7)=2.86$  Å]<sup>35</sup> and about the same as the intraring distance in  $S_8 [d(S_1 - S_3) = 3.33 \text{ Å}]^{36}$ which is not regarded as implying a significant interaction<sup>37</sup>. It is noteworthy, however, that the dithionite ion, S<sub>2</sub>O<sub>4</sub><sup>2</sup>, with an unusually long S-S distance (2.39 Å)38 dissociates in oxygenfree aqueous solution to give the SO<sub>2</sub> radical ion<sup>39</sup>, isoelectronic with  $S_3^{\pm}$ .

The probable significance of the S<sub>3</sub><sup>-</sup> radical ion (and other sulphur radical anions) in a wide variety of situations should be recognised. For example, the formation of S<sub>3</sub>. in superheated water must be considered in a discussion of sulphur transformations which occur in geothermal springs40. The discovery that sulphur radical ions are the electroactive species in the electrochemical oxidation of polysulphides is significant to battery technology, particularly for the application of Na/S<sup>0</sup> or Li/S<sup>0</sup> couples in secondary battery systems18. The formation of sulphur radical ions on the catalyst surface should be taken into account in any discussion of the mechanism of desulphurisation reactions and the reduction of SO<sub>2</sub> over metal oxides<sup>31</sup>. The well known Willgerodt-Kindler reaction provides an example of the catalytic effect of amines on the reactions of elemental sulphur<sup>41</sup> which undoubtedly involves the formation of polysulphides42, and thus, sulphur radical ions15,43.

The common feature of the wide variety of conditions in which the  $S_3^{-1}$  ion is formed is the existence of conditions in which polysulphide ions,  $S_x^2$ , are either present or can be formed easily. The trisulphur radical ion can then be produced from polysulphides either through dissociation (x = 6) or by disproportionation (2 < x < 6). As electrochemical studies<sup>13-18</sup> have shown that the reduction  $S^0 \rightarrow S^{2-}$ , or the oxidation  $S^2 \longrightarrow S^0$ , is not a single step process but occurs through polysulphide ions, the intermediacy of sulphur radical anions, for example, S<sub>3</sub><sup>-</sup> in, for instance, the biological oxidation of sulphide, is a real possibility44. Indeed, sulphur radical ions

have been invoked to explain the oxidation, by aqueous polysulphide solutions, of aryl methyl compounds to the corresponding carboxylic acids45.

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## Laser-induced orientation in macromolecular suspensions

We report here the first realisation of laser-induced changes in the orientation of macromolecules and particles in a liquid medium. We give preliminary data on the induced birefringence of two, very dilute, macromolecular suspensions; and we show that the

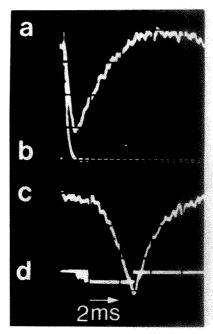


Fig. 1 Birefringence induced in Bentonite sols, at a wavelength of 488 nm. a, Laser-induced birefringence response; b, YAG laser  $(\lambda=1.06~\mu m)$  'fixed Q' pulse of approximately 8 kW maximum power; c, electrically induced birefringence response; d, a direct current electric pulse, of amplitude 31 V cm<sup>-1</sup>. Time scale, 2 ms per division; a linear detection system<sup>11</sup> was used. For b and d the same incident light intensity was used; the amplitudes of the responses are directly comparable and actually represent decreases in intensity and, thus, a negative birefringence. The sol concentration was  $1.3 \times 10^{-3}$  g ml<sup>-1</sup>.

effects accompany particle orientation rather than local heating or refractive index changes, and that they therefore indicate the foundation of a new method for characterising macromolecules.

Many molecules and particles have anisotropic optical properties. When suspended in a liquid they adopt random orientations and the overall medium is optically isotropic, until some external force field-electric, magnetic, acoustic, and so on-is applied to the suspension. Then, the anisotropic properties of the individual particles become partially impressed on the bulk medium, as the suspended particles become aligned.

Changes in the optical properties (scattered intensity), birefringence<sup>2</sup>, dichroism<sup>2</sup>, optical activity<sup>3</sup>) when pulsed electric fields are applied, have been studied often in macromolecular suspensions. The pulsed method enables molecular relaxations to be studied, whilst electric fields lead to information on the permanent dipole moments and electric polarisabilities of the suspended particles. These 'electro-optical' methods have proved particularly valuable in the study of macromolecules, because of their relatively large permanent and induced dipole moments.

It has been predicted4 and shown experimentally8 that the electric field associated with a suitably highly powered pulsed laser could provide the force field in the case of simple liquids. Similar effects have been anticipated<sup>6</sup>, but not until now realised experimentally for suspensions of macromolecules.

We assembled a conventional birefringence apparatus, with an Argon Ion, low power, continuous working laser which provided the probe light beam at a wavelength, \(\lambda\), of 488 nm. The 'orienting' beam was obtained from a Nda+ YAG laser working at a wavelength of 1.06 µm. At optical frequencies, only the electronic contributions to the electrical polarisability are effective, so that a high field is required before a suitable dipole moment can be induced in the molecules. Relatively long orientation times, from 10 µs to 1 ms, are required for the rotation of macromolecules?. To satisfy both of these requirements, the YAG laser was operated in a 'fixed Q' manner, in which the gain of the cavity was fixed and the laser was driven at a high power for a short, prefixed time; in this case approximately

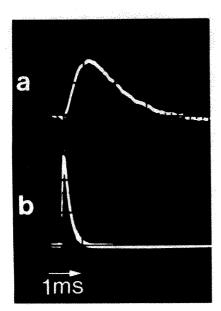


Fig. 2 Laser-induced birefringence of a solution of TRV at a wavelength of 488 nm. a, Birefringence response from TRV. Time scale, 1 ms per division. The response is an increase in light level and, therefore, a positive birefringence effect. TRV of  $3 \times 10^{-3}$  $ml^{-1}$  was studied in M/60 Sorinsens phosphate buffer at pH = 7.0. b, YAG laser 'fixed Q' pulse of approximately 8 kW maximum power.

200 us. Conventional Q switching produced pulses which were much too fast. The power of the 'fixed Q' beam was monitored through a calibrated photodiode and an approximate equivalent field strength, E, was calculated using the Poynting vector equations8. Precise values for E were not obtained, because of the complex shape of the pulse with time. It was important to operate the YAG laser in the single transverse mode, for only then was the electric vector undirectional throughout the beam cross-section. The beam was unfocused and polarised at 45° to the azimuth of the initial polariser of the birefringence optics. Using suitable dielectric coated mirrors, which transmitted the probe blue light but totally reflected the YAG infrared radiation, the orienting beam was made to travel the length of the sample cell together with the probe beam, thereby allowing a large optical cell length.

Aqueous suspensions of Wyoming sodium bentonite, a disklike clay mineral9, and a solution of tobacco rattle virus (TRV), a rod-like virus<sup>10</sup>, were studied. Figures 1a and 2a are typical of the transient birefringence traces observed. In each case the applied laser pulse is shown (Figs 1b and 2b). In order to verify that these effects arose from particle orientation, two further experiments were performed. In the case of the bentonite sol, a pulsed d.c. electric field (Fig. 1d), of suitable amplitude and duration, was applied after the laser beam. The field strength was chosen to give a similar birefringence response

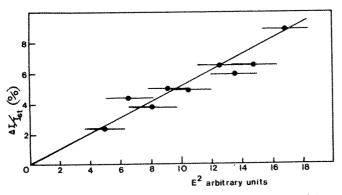


Fig. 3 Change of maximum birefringence with the square of the effective field strength, E, of the YAG laser beam. With linear detection, the light intensity change,  $\Delta I$ , is directly proportional to the birefringence, and Ist represents the residual transmitted intensity. Data for TRV.

(Fig. 1c) to that induced by the laser beam; the transient decay from both the electrically and the laser-induced effects have the same time constant, within experimental uncertainty. When interpreted as a molecular relaxation time,  $\boldsymbol{\tau},$  in the conventional manner for electric birefringence data, a value of  $\tau = 1.9$  $(\pm 0.2)$  ms was obtained. That is typical of the rotational relaxation time of that material12. With TRV, the laser-induced birefringence led to a value of  $\tau$ =1.0 (±0.1) ms, which also agrees with the value reported elsewhere13.

Rotational theory also predicts that2 the birefringence increases as the square of the electric field. Measurements were made as a function of the laser power and are presented in Fig. 3 in terms of the strength of the equivalent electric field; Fig. 3 shows the predicted quadratic dependence on E.

Future experiments will be made to quantify accurately the amplitude of the induced effects so that the electronic contribution to the polarisability can be isolated. Apart from that, and the close association with nonlinear optical effects, particle sizes can also be evaluated14 from  $\tau$ . We conclude, therefore, that these preliminary studies could lead both to a series of experiments which characterise macromolecules, and to a greater understanding of their electrical and optical properties.

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## Total reflection as derived from statistical scattering and coherent scattering theories

In the past ten years there has been much investigation of the interesting phenomenon of microscopic bubbles in liquids, particularly liquid helium, which are maintained by the presence of a light particle, such as an electron1 or positron2. In both cases, with liquid helium the bubble is believed to arise from an effective repulsive potential between bulk liquid and the light particle. Coopersmith<sup>3</sup> derived an expression for this effective potential by calculating the free energy of a light particle moving in a space having randomly distributed hard spheres.

The hard spheres move as in a classical ideal gas and statistical equilibrium between light particle and gas is assumed. Only s-wave scattering was considered. The mean free energy of the light particle is found to have a minimum energy which is due to space-limitation of its motion. The interaction free energy was shown rigorously to be

due to single scattering, all higher order multiple scattering effects being zero. In equation (1) n is the density of scattering centres,  $a_s$  the scattering length and m the light particle mass. Thus a particle of mass m striking the surface of a liquid having n molecules per unit volume from outside would require a kinetic energy at least  $k^2h^2/2m=E_F$  in order not to be totally reflected from the liquid surface. Therefore, the critical wave number for total reflection at normal incidence is given by

$$k^2 = 4\pi n a_s \tag{2}$$

Eighteen years earlier, Goldberger and Seitz<sup>4</sup> investigated coherent scattering from randomly distributed scattering centres, and produced results which have, for example, been applied to neutron refraction at liquid surfaces. According to their theory a particle of mass m will be totally externally reflected when normally incident upon a liquid surface unless

$$k^2 \geqslant 2n(\pi\sigma_s)^{1/2} \tag{3}$$

which agrees with equation (2) if we put  $\sigma_s = 4\pi a_s^2$  and take the positive square root, indicating repulsion.

So these two strikingly different approaches yield the same result in the low energy limit, for s-wave scattering.

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## Measurements of K, Rb, U, Sr and Pb in diamonds containing inclusions

KNOWLEDGE of the Pb and Sr isotopic compositions of syngenetic inclusions in diamonds is important for several reasons. As diamonds probably crystallised at depths of more than 100 km, the isotopic data may provide constraints on the mode of the geochemical evolution of the Earth. It may also be possible to test assumed U/Pb ratios  $(U/Pb=\mu)$  for the mantle and to estimate the age of particular diamonds. Diamonds are particularly appropriate for these studies because, in contrast to kimberlites and their associated nodules, any contamination by crustal material can be overcome by washing in acid.

Here we report preliminary measurements of the K, Rb, Sr, U and Pb concentrations in diamonds. As far as we know the only other reported measurements are by Fesq et al.1 who used instrumental neutron activation analysis to determine the concentrations of Sr, Rb and K. They found that these elements are far more abundant in diamonds with visible inclusions than in those apparently free of inclusions.

We investigated two batches of diamonds from the Premier Mine, South Africa, one containing green (mainly diopside) and the other black (mainly sulphide) inclusions. The 'green' diamonds (22 stones, weighing 0.2674 g) were analysed for Sr, Rb and K, and the 'black' diamonds (27 stones, weighing 0.3188 g) for Pb and U. The diamonds were small, 1-2 mm in size (in general, inclusions are of roughly constant size, so for a given weight of diamonds the percentage occupied by the inclusion is highest in small stones). The diamonds were colourless or brown, of good crystal morphology and free from surface fractures.

Diopside is a fairly common inclusion in Premier Mine diamonds, occurring as pale green euhedral or anhedral crystals. The diopsides chosen were free from associated black material<sup>2</sup> although in some cases internal fractures were present. The sulphide occurrences in diamond are more varied2, and sulphides occurring in internal rosette fractures associated with colourless inclusions, and as discrete sulphide inclusions were chosep. The sulphides probably consist mainly of pyrrhotite2, but the chemistry was not determined. They were distinguished from graphite by colour and lustre3. The percentage by weight that the inclusions occupied within both batches was roughly estimated as 0.1%.

The Premier pipe is older than 1,115±15 Myr and younger than  $1,954 \pm 30$  Myr (refs 4 and 5). It contains at least four distinct intrusions of kimberlite with ages that may range from 1,200-1,400 Myr, but it could not be ascertained to which type our diamonds belonged. The best dated type of kimberlite (Type 1) has a Rb/Sr age of 1,250 ± 20 Myr (H. L. A. unpublished), so we assumed that age in our work, but the assumption of any age in the range indicated would not affect significantly our tentative conclusions.

For each experiment we used only 10 g of reagent prepared by triple distillation in quartz under sub-boiling point conditions (in the case of H<sub>2</sub>O and HCl); HF and HNO<sub>3</sub> were prepared as in ref. 6.

The diamonds were cleaned by prolonged and repeated heating and ultrasonic agitation in HF, HClO, and water, and then burnt over a period of about 15 min in a clean, covered quartz crucible at ~ 850°C, in a flow of highpurity filtered oxygen. A preliminary experiment using a platinum crucible gave a Pb blank much higher than the quartz; but even the quartz (or possibly the oxygen) still constitutes a serious source of Pb contamination. The crucible, about 15 cm long, had a water-cooled ring near the top to condense volatilised molecules.

The residue was transferred to a teflon autoclave using dilute acid. After HF was added it was heated at 200° C for 6 h. After evaporation the residue dissolved readily in dilute HCl. This solution was then aliquoted for three separate mass spectrometer analyses: isotopic composition (of Sr and Pb); concentration of K, Rb (jointly) and U; and concentration of Sr and Pb.

Concentrations were determined by isotope dilution. Isotopic tracers of high purity (>99%) were used in all

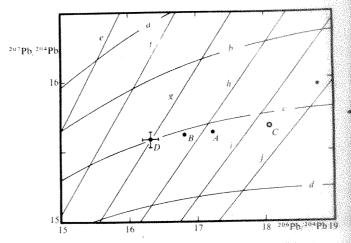


Fig. 1  $^{207}\text{Pb}/^{204}\text{Pb}$  and  $^{206}\text{Pb}/^{204}\text{Pb}$  data plotted on a lead growth diagram.  $a, \mu = 11; b, \mu = 10; c, \mu = 9; d, \mu = 8; e, T = 2,500 \text{ Myr}; f, T = 2,000 \text{ Myr}; g, T = 1,500 \text{ Myr}; h, T = 1,000 \text{ Myr}; i, T = 500 \text{ Myr}; j, T = 0. A, Experimental data corrected only for mass spectrometer fractionation; B, results from correction for the blank, the isotopic composition of which is$ indicated by C; D, results from the additional correction for radiogenic Pb. Parameters used:  $a_0 = 9.56$ ;  $b_0 = 10.42$ ;  $\lambda^{238} = 0.1537 \times 10^{-9} \text{ yr}^{-1}$ ;  $\lambda^{235} = 0.9722 \times 10^{-9} \text{ yr}^{-1}$ .

Table 1 Results on inclusions in Premier diamonds

	K (ng)	Rb (ng)	Sr (ng)	K/Rb	87Rb/86Sr	<sup>87</sup> Sr/ <sup>86</sup> Sr	U (ng)	Pb (ng)	<sup>238</sup> U/ <sup>204</sup> Pb	<sup>206</sup> Pb/ <sup>204</sup> Pb	<sup>207</sup> Pb/ <sup>204</sup> Pb	<sup>208</sup> Pb/ <sup>204</sup> Pb
Reagent blank (2 g HNO <sub>3</sub> + 2 g HF + 6 g H <sub>3</sub> O)	20.9	0.102	0.398				0.239	0.62				
First full blank on method (10 g reagent)	65.0	0.128	0.339				0.402					
Final full blank on method (10 g reagent)  Average of blanks on method 'Green' pyroxene inclusions	40.7	0.106 0.117 2.866	0.529			0.7053* +0.0007	0.385	13.60 12.87				
% Blank 'Black' sulphide inclusions % Blank	6.8	4.1	0.88			200700	1.529 25.2	40.22 32.0		17.24† ±0.06	15.64† ±0.05	37.05† ±0.20
Inclusion results — blank on method	558.7	2.749	59.36	203.2	0.135	0.7053 ±0.0007	‡ 1.144	27.35	2.473	$^{16.84\$}_{\pm 0.06}$	$^{15.62\$}_{\pm 0.05}$	$^{36.24\$}_{\pm 0.20}$
Age-corrected isotopic ratios¶					•	±0.7029 ±0.0007				16.32 ±0.12	15.58 ±0.06	

• Normalised to  ${}^{88}Sr/{}^{86}Sr = 8.375$ .

Corrected for mass-spectrometer fractionation to NBS 981 standard. Corrected for blank 87Sr/86Sr = 0.7090.

All quoted errors are estimated 10 values.

cases except U, and the sensitivity was accordingly good for all elements except U. The accuracy of the concentration data is limited in all cases (again except U) by the blank levels and not by the precision of the mass spectrometer measurements. Potassium, rubidium and lead were run on single filaments by the silica-gel method, and Sr and U were run on double filaments. The decision to make no chemical separations of the elements under consideration had deleterious effects in both the Sr and Pb isotopic composition analyses. With Sr, Rb interference "Rb/8"Sr~2%) was a problem, but correction using \*\*Rb/\*\*Rb=2.600 was considered reliable as no samples enriched in 87Rb have even been measured on the mass spectrometer in question. With Pb, although test runs with pure, 50 ng, Pb samples gave satisfactory ion beams, the diamond sample yielded only a small beam (204Pb~1-3 mV), and this severely limited the precision.

Table 1 shows the results obtained. The blanks of Sr, K and Rb (both on the reagents and on the full procedure) are of an acceptably low level, but the blanks of U and Pb amounted to 25% and 32% of the sample, respectively. The U tracer used is not sufficiently sensitive for such low level work, and the blank levels may well have been overestimated. Only in the case of Pb is the full procedure blank significantly greater than the reagent blank. The most significant result is that ~ 30 and 60 ng of Pb and Sr,

respectively, are contained in the relatively modest quantities of diamonds used. On the evidence of Fesq et al.1 and our own unreported data from a preliminary experiment on a batch of diamonds with only graphite inclusions, we believe that this Pb and Sr is contained in the inclusions; using the very rough abundance estimates of the inclusions, the concentrations are ~ 100 p.p.m. of Pb in the sulphides and  $\sim 200$  p.p.m. of Sr in the diopsides. But even if the Pb and Sr is contained in the diamond structure rather than in the inclusions, our conclusions relating to the isotopic data will not be affected. The K/Rb ratio of the diopside inclusions is ~ 200, a similar value to that obtained for periodotite nodules and their constituent diopsides8.

The Pb and Sr isotopic ratios were corrected in several ways. The \*7Sr/\*6Sr ratio was normalised to a \*\*Sr/\*6Sr ratio of 8.375; if the 87Sr/86Sr ratio of the contamination Sr was 0.7090, the blank correction is negligible; correction for the radiogenic component was made on the basis of the measured Rb/Sr ratio and an age of 1,250 Myr. The 207Pb/ <sup>204</sup>Pb and <sup>206</sup>Pb/<sup>204</sup>Pb ratios were arbitrarily corrected for fractionation by comparing measured and accepted values on the NBS 981 standard Pb. Correction for the radiogenic Pb was made using the measured U/Pb ratio, and because of the large uncertainty in this ratio, the error on the isotopic ratios has been increased arbitrarily. The influence of the isotopic composition of the contaminating Pb is in

Table 2 Rb, Sr and 87Sr/88Sr values for Premier carbonatites

	1 able 2	Ro, Si anu	31/ 31 value	3 IOI I I WING
Sample	Rb (p.p.m.)	Sr (p.p.m.)	<sup>87</sup> Sr/ <sup>86</sup> Sr	Remarks
PC/B PC/2 PC/3	0.32 1.48 0.85	1,042 838 1,579	0.7025 0.7032 0.7044	High Mg-brucite Si-rich kimberlitic carbonatite A complex rock, predominantly calcite

 $<sup>^{87}</sup>$ Sr/ $^{88}$ Sr corrected to  $^{88}$ Sr/ $^{86}$ Sr = 8.375; accuracy in  $^{87}$ Sr/ $^{86}$ Sr estimated as  $\pm$  0.0003. Using an age of 1,250 Myr the correction for radiogenic Sr is negligible. Three distinct types of carbonatite were sampled, and all samples were petrographically assessed to be fresh. Sample PC/3 contains, however, two generations of calcite and some of the calcite may be crust-derived; that would account for the higher 87Sr/86Sr ratio and this result is rejected.

Using measured blank lead isotopic composition 206/204 = 18.08; 207/204 = 15.68; 208/204 = 38.77. ¶ Using age of Premier pipe of 1,250 Myr; error for Pb ratios arbitrarily increased to take account of uncertainty in U/Pb ratio;  $\lambda^{Rb} = 1.39 \times 10^{-11} \text{ yr}^{-1}$ .

this case large, and so an approximate measurement of this parameter was made by combining the Pb ( $\sim 25$  ng) from two blank runs.

The diagram shows the corrected and uncorrected Pb isotopic ratios in relation to Pb growth curves. In view of the limited nature of the data these results must be treated with caution, but it seems that the  $\mu$  value for the source region of these diamonds is close to 9.0 and that if a single-stage model is assumed, the age is close to 1,500 Myr. Whether this age refers to the time of pipe formation (assuming that isotopic equilibrium was maintained between Pb in possibly older diamonds and in the surrounding mantle until pipe formation), or to the time of diamond formation itself, is unknown. Further data are needed; a prime objective of such work would be to detect possible mantle depletion in the lithophile elements, as reflected by changing  $\mu$  values with time.

A \*7Sr/\*\*Sr ratio for carbonatite from Premier, of  $0.7028 \pm 0.0003$  has been reported, and the results of three further measurements are listed in Table 2. The kimberlite found at Premier is considered as too altered to warrant analysis (see ref. 10), but the fresher carbonatites, if derived from an original kimberlite magma rich in carbonates, should have the same \*7Sr/\*6Sr ratio as the original kimberlite. The fact that the corrected 87Sr/86Sr ratio for Premier diamonds (0.7028 ± 0.0006) is almost identical with the results listed here for carbonatites from the same source, suggests a genetic relationship between diamonds and the original kimberlite magma. The observed \*7Sr/\*\*Sr ratio is lower than the main trend for basic rocks from southern Africa5; layering of the mantle is implied, with the deep-seated source region of the diamonds characterised by low \*7Sr/\*\*Sr ratios.

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### Skull of Palaechthon nacimienti

Species in or near the ancestry of living primates first appear in the late Cretaceous and early Palaeocene of North America. Subsequent adaptive radiation of the Purgatorius-like ancestral stock produced the plesiadapoid families (Plesiadapidae, Carpolestidae, Paromomyidae) of the middle and late Palaeocene. Specialised members of all three families survived into the early Eocene, the paromomyid genus Phenacolemur persisting into the late Eocene. Most of the plesiadapoid species are known only from incomplete dentitions. In 1948, a crushed but nearly complete skull of a paromomyid was recovered from strata of middle Palaeocene age in the Kutz Canyon area of the San Juan Basin, New Mexico. The specimen has been described by Wilson and Szalay1, who assign it to a new species (P. nacimienti) of the genus Palaechthon, known also from the mid-Palaeocene of Montana and Wyoming. The loss of the upper and lower first premolars excludes P. nacimienti from the ancestry of some of the Eocene prosimian lineages. Nevertheless, its persistently primitive molar morphology suggests that it may more closely resemble the last common ancestor of the plesiadapoids and the Eocene primates of modern aspect than do other plesiadapoids for which cranial remains are known. Skulls or partial skulls are known for dentally more specialised genera of each plesiadapoid family: Plesiadapis (Plesiadapidae), Carpolestes (Carpolestidae), and Phenacolemur (Paromomyidae).) We present here a reconstruction of the skull of P. nacimienti, together with some preliminary functional interpretations of its cranial and dental anatomy.

The skull is unevenly crushed in the dorsoventral plane and lacks the zygomata and the anterior extremity of the rostrum. Its caudal end is missing, including the greater portion of the petrosals and basioccipital, the occipital condyles and foramen magnum, and the corresponding portions of the skull roof. The basicranium is badly fragmented, and foramina cannot be identified with certainty. The ear region is represented only by dubious fragments of the anterior tip of the petrosal; the bulla is completely lost. (The original description of this speciment stated that "... there are signs that a sizable bulla was present," and that "the pterygoid crests are only 0.7 mm apart". The authors may have mistaken fragments of the widely-divergent pterygoid laminae for fragments of the bullae.) Relative brain size cannot be estimated from the preserved material.

Each of us attempted an independent reconstruction of the skull. The results differed in only a few details, which were resolved in preparing Fig. 1. Linear measurements of the skull

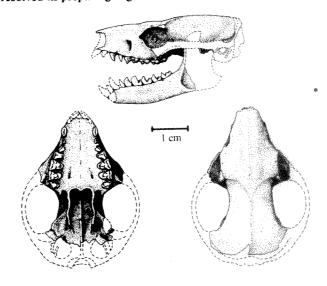


Fig. 1 Ventral, lateral, and dorsal views of the skull of *Palaechthon nacimienti*, based principally on Univ. Kansas Nat. Hist. Mus. No. 9557 and No. 9559. Specimen UKMNH No. 7903, which was originally included in the hypodigm of *P. nacimienti*, represents a new species and accordingly was omitted from consideration here.

and teeth were compared with similar data for extant prosimians, tree shrews, elephant shrews, dermopterans, squirrels, didelphids and phalangerids, as an aid in assessing the adaptive significance of the fossil's morphology.

The cheek teeth of *Palaechthon* show a constellation of features similar to that seen in the extant insectivorous prosimians *Loris*, *Arctocebus*, *Tarsius*, *Galago demidovii* and *Galago senegalensis*. The molars are relatively large and heavily worn. The lower molar crowns of *Palaechthon* are relatively high, and exhibit pronounced concavely-curved leading edges of wear surfaces 1-4. (These and related terms used here are defined in ref. 2.) In mastication, these edges were moved dorsally and anteromedially past matching pairs of reciprocally-curved upper molar blades. The leading edges of upper and lower wear surfaces 5 and 6 are also long. These features are correlated with shearing functions and resistance to wear, and are reduced in more frugivorous extant prosimians<sup>3</sup>.

In Palaechthon, the origin of the anterior temporalis muscle extends forward to a point above the posterior upper molar. The faint temporal lines converge to form a midsagittal line which extends over approximately the posterior 70% of the temporal fossa. The linea obliqua of the mandible, which strengthens the coronoid process against the pull of the temporalis and provides an insertion for the anterior and zygomatic parts of that muscle, is robust and in high relief relative to the masseteric fossa behind it. These features suggest that the principal axis of the temporalis fibres was nearly vertical with respect to the occlusal plane, and situated more anteriorly than is typical of extant prosimians. Similar features are seen among rodents, and are pronounced in a larger and later plesiadapoid from Europe, Plesiadapis tricuspidens1. The origin of the superficial masseter is marked by a crest on the ventral surface of the orbital margin, above the buccal edge of the second upper molar. This origin is farther forward than in most extant prosimians, and implies a more horizontal orientation for this muscle, as in lorisids.

The medial and lateral pterygoid laminae diverge widely, enclosing a large pterygoid fossa. If the origin of the medial pterygoid was restricted to this fossa, as it is in some extant members of the Insectivora, it was probably relatively larger than in extant prosimians of comparable size. If this muscle also had a superficial head arising from the medial orbital wall, as in Galago, Tarsius, Tupaia and Felis, it must have been exceptionally large. Palaechthon's well-developed anterior temporalis, enlarged medial pterygoid and horizontally oriented superficial masseter may be in part related to the use of the anterior lower incisors in protrusive prying, shovelling or other ingestive movements.

The orbital margins are deformed but largely preserved. The supraorbital shelf is well developed, and a short superior postorbital process is present. The shape of the superior orbital margin indicates that a complete postorbital bar was probably absent. The dihedral angle between orbital and midsagittal planes (measured from a sculpted reconstruction) was about 35°, and the line of intersection of these two planes must have been approximately parallel to the skull roof. In these respects, *Palaechthon* resembles terrestrial sciurids<sup>4,5</sup>. Visual-field overlap could not have been extensive.

The orbital diameter of *Palaechthon* was approximately 7 mm. This is smaller than would be expected for extant prosimians, tree shrews, elephant shrews, sciurids, dermopterans or arboreal didelphids of comparable body size. Nocturnal terrestrial foragers like *Echinosorex* and *Monodelphis* resemble *Palaechthon* in relative orbital diameter.

The interorbital breadth of *Palaechthon* is unusually great. In extant mammals, three principal factors influence this dimension. First, in animals with relatively gracile zygomata, orbital convergence necessitates reduction of interorbital breadth<sup>6</sup>. Second, interorbital breadth may be increased for the transmission of exceptional compressive stresses generated in mastication or incisal biting, as in *Daubentonia*, *Dactylopsila*<sup>7</sup>,

Alouatta<sup>8</sup> and colobines. Third, interorbital breadth may increase in conjunction with an enlargement of the olfactory fossa, as in Nasua. The last two of these factors probably operated in Palaechthon; the preserved parts of the skull demonstrate the resulting lateral position of the orbits (accentuated by the supraorbital shelf) and the large olfactory fossa.

Compared with extant prosimians and tupaine tree shrews, *Palaechthon* has a large infraorbital foramen, implying that the innervation and blood supply of the rhinarium and vibrissal roots were correspondingly well developed. The relative size of this foramen resembles that of living hedgehogs and carnivores.

In summary, the known cranial and dental remains of *P. nacimienti* imply that it relied more on tactile and olfactory information than on vision in its foraging activities. The well-developed anterior temporalis suggests that powerful occlusal forces could be exerted in mastication. The molar morphology indicates a predominantly insectivorous diet. An adaptive mode involving substantial amounts of nocturnal foraging on the forest floor is consonant with the observed morphology. Squirrel-like arboreal habits involving diurnal foraging for plant foods, or visually-directed predatory habits like those of many small marsupials or extant insectivorous prosimians, seem less likely.

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## ADP binding to relaxed scallop myofibrils

CALCIUM regulates muscle contraction in all species which have been studied. Muscle relaxes in the absence of calcium and contracts when it is present in micromolar concentrations<sup>1-3</sup>. Contraction is caused by the interaction of myosin with ATP and with the actin of the thin filament, and the effect of calcium is to modulate this interaction<sup>1-8</sup>. Although these features apply to all muscles, the location of the calcium binding and thus the mechanism of its action differs in different phyla; regulation may be associated with the myosin, or the thin filaments or both<sup>2,3,7</sup>.

The mechanism of the myosin ATPase in vertebrate muscle, in which regulation is thin filament-linked, has been widely studied. Myosin rapidly binds and hydrolyses ATP to form a myosin·ADP·P<sub>1</sub> intermediate<sup>9,10</sup>. The breakdown of this complex is the rate limiting step of hydrolysis

and actin accelerates this rate<sup>11</sup>. Therefore, in relaxed vertebrate muscle where the actin-myosin-ADP-P<sub>1</sub> interaction is blocked by the troponin-tropomyosin of the thin filaments, ADP and P<sub>1</sub> are found bound to myosin in the steady state<sup>12</sup>.

In molluscs, on the other hand, calcium regulation is myosin-linked and it is not known whether, in the absence of calcium, the hydrolysis of ATP, itself, is blocked and myosin ADP P<sub>i</sub> is not formed, or whether myosin ADP P<sub>i</sub> is formed but its association with the thin filaments is blocked. In this study we show that in molluscs, as in vertebrates, ADP is bound to myosin in relaxing conditions.

Myofibrils were prepared from both fresh and glycerinated Aequipecten irradians striated adductor muscles. Muscles were glycerinated according to Kendrick-Jones et al.7 in a solution consisting of 50 mM NaCl, 1 mM MgCl<sub>2</sub>, 5 mM phosphate buffer (pH 7.0), 0.1 mM sodium azide and 50% glycerol, and stored at  $-20^{\circ}$  C for one month. To prepare myofibrils, muscles were homogenised in a Sorvall Omni mixer in 5 mM piperazine-N, N'-bis-2-ethanesulphonic acid (PIPES), pH 7.1, 5 mM ethyleneglycol-bis (β-aminoethyl ether)-N,N'-tetraacetic acid (EGTA), pH 7.0, 50 mM KCl and 5 mM MgCl<sub>2</sub>, and sedimented at 12,000g for 10 min. The pellet was rehomogenised in the same solution and resedimented at 500g for 10 min twice. A homogenous suspension of myofibrils, containing 15-20 mg protein ml-1, was embedded in a 1% agarose gel as previously described<sup>12,13</sup>. Blocks of the gel (100 to 200 nl) were cut out and incubated at 2° C in relaxing solution containing 14C-ATP at 200 mCi mmol<sup>-1</sup>; <sup>14</sup>C-mannitol was used as a volume

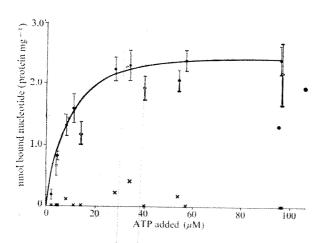


Fig. 1 Nucleotides bound by scallop striated muscle in relaxing conditions in the presence of different concentrations of MgATP. Conditions: 5 mM PIPES buffer; 5 mM EGTA; 5 mM MgCl<sub>2</sub>; 50 mM KCl; 4 mM creatine phosphate; phosphocreatine kinase (5 mg ml<sup>-1</sup>). 1 mM sodium azide 0-100 μM <sup>14</sup>C-ATP, <sup>14</sup>C-mannitol, pH 7.1, i=0.095, 2° C. ADP bound to glycerinated, ♠, and fresh, ○, myofibrils; ×, corresponding ATP binding; vertical bars, ± 5% fiducial limits of eight assays.

marker and an ATP regenerating system was included in the medium (Fig. 1). After 3 min, the gel blocks were transferred to trichloroacetic acid and the bound nucleotide content was determined. The details of this procedure have been described in a previous paper  $^{12,13}$ . The myofibrillar ATPase (that is, Mg-ATPase) was measured in a pH stat as previously described at  $2^{\circ}$  C and  $25^{\circ}$  C.

In relaxing conditions (ATP, no calcium) where the Mg-ATPase was at least 97% inhibited, myofibrils from glycerinated scallop striated muscle bound a large quantity of ADP and only minimal amounts of ATP and AMP (Fig. 1). Assuming a simple binding, a nonlinear regression analysis

of the data<sup>14</sup> showed a maximum ADP binding of 2.69±0.15 nmol per mg protein, and the dissociation constant was 9 mM±2 s.e. The amount of ADP bound was almost identical to the estimated quantity of myosin sites in the muscle (2.75 nmol mg<sup>-1</sup> based on a 62% myosin content<sup>8</sup> and a weight of 225,000 daltons per active site<sup>13,18</sup>). Myofibrils from fresh muscle were about 90% calcium sensitive, bound slightly less ADP (2.45±0.15 nmol mg<sup>-1</sup>) and had a slightly higher dissociation constant (12±3 mM). The smooth ('catch') muscle of Aequipecten, which has a smaller myosin content than the striated muscle, also bound only ADP in relaxing conditions, with a maximum binding of 0.75 nmol per mg protein.

These results show that the steady-state intermediate of relaxed myosin ATPase of scallop muscle is M-ADP (presumably the P<sub>1</sub> moiety of ATP is also bound in this complex<sup>9,10,12</sup>); thus, the decomposition of this complex is rate limiting. It follows that calcium activation cannot act at the hydrolytic step: M·ATP=M·ADP·P, as this reaction is not rate limiting, but must affect the subsequent reaction of M·ADP·P<sub>1</sub> with actin. It was previously shown that scallop myosin has a reduced apparent affinity for actin in relaxing conditions; in this paper the cause of this has been explained. From the results of nucleotide binding experiments on vertebrate, insect, and molluscan muscle, it seems likely that calcium regulates the actomyosin ATPase cycle at the same step in each, even though the control site may be located on myosin as in scallop7, on the thin filaments as in vertebrates1.2,23,12, or on both the myosin and the thin filaments as in insects, such as Lethocerus3,12,17

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## In vitro activity of cells from genetically lethal embryos of Drosophila

MUTATIONS that affect particular embryological events can help to elucidate the developmental interactions and cellular and biochemical mechanisms. In *Drosophila* much of the work<sup>1,2</sup> in developmental genetics has been done on strains that carry chromosomal aberrations, particularly deficiencies; or on strains that segregate aneuploid progeny, such as X-linked stocks.

Deep orange (dor) is an X-linked (1-0.3) recessive mutant
• of D. melanogaster<sup>3</sup>. In addition to an alteration in pteridine
pigments of the eye<sup>4</sup>, dor causes a peculiar type of sterility<sup>3-6</sup>.
When dor females are mated to dor males, all offspring die
during the course of embryonic development. When heterozygous females are mated to dor males, however, all of the
expected progeny types survive and develop normally. This
indicates that the viability of dor embryos depends on the
maternal as well as the zygote genotype.

Thus dor embryos survive only if an essential developmental component is provided by the egg cytoplasm produced by heterozygous mothers. This repair of dor egg defect by the essential component has been recently confirmed by an injection experiment in which cytoplasm from unfertilised normal eggs was injected into dor embryos at the syncytial preblastoderm stage of development? The present experiment reveals that some types of cells from dor embryos proliferate actively and manifest their function for prolonged periods when they are cultured in a chemically defined medium with some macromolecular supplementations, especially when the wild-type egg extract was added to culture medium.

The dor strain used was a balanced stock carrying C1B X chromosome. Eggs collected from matings of dor/dor females and dor/Y males were dechorionated by a 6-min treatment with 3% sodium hypochloride. The eggs were then rinsed with distilled water and transferred to physiological salt solution, in which they were allowed to develop at 25° C. The developmental stages of dechorionated eggs were readily determined through the transparent vitelline membrane under a binocular microscope. Among 463 eggs obtained from the above matings, 19 (4.1%) died before gastrulation, 179 (38.7%) died by abnormal gastrulation, 204 (44.0%) died after the stage of sac-like midgut, 61 (13.2%) died after muscular movement and none hatched.

Dor embryos which developed beyond gastrulation were sterilised in 70% ethyl alcohol for 10 min, rinsed three times with sterile salt solution, and used for in vitro cultivation. The procedure for cultivation of Drosophila embryonic cells has been previously described. Embryos were torn into small fragments with a pair of fine needles and cultured in T-5 flasks in medium K-17 (ref. 9) supplemented with 0.1 mg ml<sup>-1</sup> fetuin and 15% foetal bovine serum.

After several hours of cultivation, spindle-shaped muscle cells came out from the cut ends of tissue fragments (Fig. 1a).

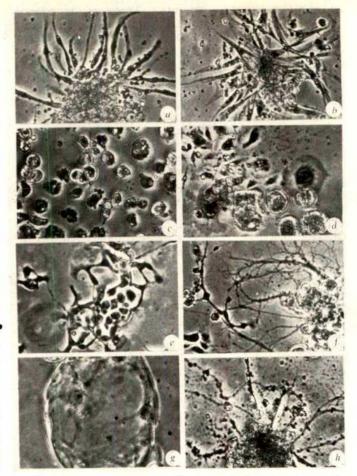


Fig. 1 Various types of cells from dor lethal embryos of Drosophila melanogaster (×450). a, Muscle cells after 4-d cultivation; b, muscle cells pulsating synchronously after 34-d cultivation; c, epithelial cells with many cytoplasmic granules after 3-d cultivation; d, epithelial cells showing chitinous pigmentation after 7-d cultivation; e, fibroblastic cells after 3-d cultivation; f, nerve cells with network of nerve fibres after 8-d cultivation; g, a cellular sphere after 10-d cultivation with wild-type egg extract; h, nerve fibres with deposits of small droplets on them after 10-d cultivation with wild-type egg extract.

They were 50–100 µm in length and had an ovoid nucleus. They gradually increased in number around the original tissue fragments. The formation of syncytial complexes, which has been observed in cultures of wild-type embryonic cells, was not found in cultures of dor embryonic cells. Some of these muscle cells pulsated at a particular interval for each cell. When they were in contact with each other, they pulsated synchronously. This pulsation continued for more than 5 weeks under culture conditions employed (Fig. 1b).

Flat polygonal epithelial cells were also found at the initial

Call time	Growth and differentiation	Oregon-R <sup>8</sup>	Embryonic cells from dor without	dor wit
Cell type	Growth and differentiation	O. Ugon It	OEE*	OEE
Muscle cells	Pulsation	+	+	+
ividscie eens	Syncytium formation	+	<del>-</del>	+
Epithelial cells	Monolayer sheet	+	±	+
Epithenai cens	Maturation	+	+	+
Fibroblastic cells	Monolayer sheet	+	+	+
Cellular spheres	Sphere formation	+	-	+
Small cells	Monolayer sheet	+	-	-
Small cens	Regular arrangement	+		- 170
Nerve cells	Fibre extension	+	+	+
Nerve cens	Fibre branching	+	+	+
	Droplet formation	+		+

<sup>\*</sup> Oregon-R egg extract.

stage of cultivation. They grew slowly and increased gradually in size with many cytoplasmic granules (Fig. 1c). It seemed that their maturation proceeded. After cultivation for 7 d a chitinous pigmentation (coloured brown) was observed in the cytoplasm of some cells (Fig. 1d).

Fibroblastic cells which were most motile appeared after 3-d cultivation (Fig. 1e). They migrated rapidly by extension and contraction of their cytoplasmic processes. In cultures of wild-type embryonic cells it has been found that fibroblastic cells formed baloon-like cellular spheres8. In the present cultures of dor embryonic cells, however, no such cellular spheres were observed.

Some nerve fibres extended from nerve cells. They increased in length and formed many branches. After cultivation for 8 d nerve fibres made contact with each other and formed a nerve network (Fig. 1f). In further cultivation, however, the deposition of small droplets, which has been found in cultures of wild-type embryonic cells and seems to be some secretion from the nerve fibres, were not observed in cultures of dor embryonic cells.

In cultures of wild-type embryonic cells, some groups of extremely small cells which were epithelial in morphology, about 3 µm in diameter and seemed to be imaginal disk cells has been observed. In the cultures of dor embryonic cells, no such small cells were detected in identical culture conditions.

The characterisation and phenotypic expression of cell types appearing in cultures of dor embryonic cells are summarised in Table 1. In this table, the findings in cultures of wild-type embryonic cells are also listed for comparison. This indicates that mesodermal cells such as muscle cells and fibroblastic cells from dor embryos were maintained in a functionally active state for a relatively extended period over the prospective lethal phase of the dor embryos, although some defects in properties such as syncytium formation of muscle cells were found. On the other hand, ectodermal cells such as epithelial cells, small cells, and nerve cells grew scarcely and manifested only a part of their respective behaviour and functions in the in vitro conditions employed.

Unfertilised eggs collected from the wild-type strain, Oregon-R, were homogenised in culture medium and centrifuged at 2,000 r.p.m. for 15 min. When dor embryonic cells were cultured in medium containing the wild-type egg extract (100 eggs ml-1) obtained by this method, defects in behaviour and functions of ectodermal cells were found to be almost repaired (Table 1); syncytium formation of muscle cells, formation of cellular spheres (Fig. 1g), and droplet formation on nerve fibres (Fig. 1h). Even in cultures with the wild-type egg extract, the growth and formation of regular arrangement of small cells were still not observed.

Since the dor mutant is known to be abnormal in pteridine metabolism, some pteridines may have an effect on the normal growth and differentiation of dor embryonic cells. The procedure for in vitro cultivation of dor embryonic cells seems to offer an experimental approach to analysing the effective substances which are produced by the normal allele of dor gene and may be necessary for normal development.

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## Is calcium ionophore a universal activator for unfertilised eggs?

We have previously exposed sea urchin eggs to micromolar amounts of the divalent transporting ionophore A23187, and observed every parameter of normal fertilisation. The cortical reaction with elevation of the fertilisation membrane, the plasma membrane conductance changes, the respiratory burst, and increases in protein and DNA synthesis were all initiated in the usual fashion. These activations of Lytechinus pictus and Strongylocentrotus purpuratus eggs were independent of the ionic composition of the external solutions<sup>1</sup>. A23187 seemed to act by releasing intracellular Ca2+, for eggs preloaded with 45Ca showed a twentyfold increase in 45Ca-efflux when activated by ionophore or fertilisation<sup>1</sup>. Measurements of free and bound calcium and magnesium in homogenates of unfertilised eggs showed that most of the Mg2+ was already available in soluble form, whereas the Ca2+ was sequestered but available for release<sup>1</sup>. After we found that 50 µM A23187 could activate eggs of the mollusc Acmaea insessa and the protochordate Ciona intestinalis to undergo several abnormal cleavages, we wondered whether ionophore activation might be a general phenomenon. To our knowledge artificial parthenogenesis has not been obtained with Acmaea and Ciona eggs by other methods. We have now found that calcium ionophore activation is common to several species widely separated phylogenetically.

We used three species whose egg activation has been well described in the literature; the bat-star, Patiria miniata (Asteroidea), the toad, Xenopus laevis (Anura), and the hamster, Mesocricetus auratus (Mammalia). A 5 mM stock solution of A23187 (obtained from R. L. Hamill, Eli Lilly Co., Indianapolis) was prepared in dimethylsulphoxide (DMSO) and stored in the dark. Appropriate controls were run with DMSO and indicated no effect. When the stock ionophore solution was diluted for use with occutes, the solutions were stirred continuously to ensure good mixing. since the ionophore is very insoluble in water and there is some precipitation. Final concentrations given here are therefore maximum estimates.

Isolated ovaries of P. miniata were teased to free the operates. which were stored as premeiotic cells in filtered seawater at 16° C. When oocytes were first incubated in the hormone 1-methyladenine (1-MA)  $1\times10^{-5}M$  for 30 min to induce maturation<sup>2-4</sup>, exposure to ionophore (5-25 μM) initiated a cortical reaction indistinguishable from that initiated by sperm.

Meiosis continued in these activated eggs and two polar bodies were produced as in eggs treated with 1-MA only. Usually, the activated eggs did not divide. After 20 h more than 90% were still one cell. One two-cell and one 12-16-cell stages were seen.

Ionophore activation did not depend on exogenous calcium. Eggs pretreated with 1-MA could be activated by 5-25 µM ionophore in Ca2+-free seawater (Na+ substituted for Ca2+ plus 2 mM ethylene glycol bis(β-aminoethyl ether)N,N,N'N'tetraacetic acid (EGTA)).

X. laevis females were treated as described by Wolf and Hedrick<sup>5</sup>, to obtain eggs, which were expressed into De Boer's solution (0.11 M NaCl, 0.0013 M KCl, 0.00440 M CaCl<sub>2</sub> plus solid NaHCO<sub>3</sub> to pH 7.2) and 5-10 oocytes per ml were placed into small syracuse dishes. Criteria of activation

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Table 1 Activation of X. laevis eggs by A23187								
*Solution	No. activated	No. treated	% activated*					
5μ M A23187 in 0.1% DMSO-De Boer's	13	15	87					
10 μM A23187 in 0.2% DMSO-De Boer's	21	25	84					
De Boer's	0	5	0					
0.1% DMSO-De Boer's	0	18	0					
0.2% DMSO-De Boer's	0	25	0					
5 μM A23187+ Ca <sup>2+</sup> -free De Boer's +2 mM EDTA	10	10	100					
Ca <sup>2+</sup> -free De Boer's +0.1% DMSO	0	6	0					

<sup>\*</sup>Percentage observed with both cortical reaction and pigment migration.

were first, elevation of a fertilisation membrane and, second, contraction or capping of pigment from vegetal to animal hemisphere.

As Table 1 shows, 5-10 µM A23187 elicited both cortical reactions and pigment migration within 2-3 min: both changes were complete by 5-13 min. These times are similar to those observed after normal fertilisation. The same activations were observed in Ca<sup>2+</sup>-free De Boer's medium supplemented with 2 mM ethylene-diaminetetraacetic acid (EDTA).

Mature unfertilised eggs of *M. auratus* were obtained from oviducts of unbred females 15-17 h after injection of human chorionic gonadotrophin<sup>6</sup>. The eggs were freed from surrounding cumulus cells by treatment for 15 min (25° C) with 0.1% bovine testicular hyaluronidase (300 USP U ml<sup>-1</sup>; Nutritional Biochemicals) in Biggers, Whitten and Whittingham (BWW) medium and rinsed thoroughly with Ca<sup>2+</sup>-Mg<sup>2+</sup>-free BWW. (The medium was modified by replacing albumin with 0.2% polyvinylpyrrolidone. *pH* was adjusted to 7.3 by addition of a small amount of N/5 HCl.)

The eggs were placed in 0.2 ml of either normal or Ca<sup>2+</sup>-Mg<sup>2+</sup>-free BWW (pH 7.3) containing freshly diluted 3–20 μM A23187 and incubated under mineral oil (Squibb) in a watchglass in an air atmosphere at 37°-38° C. At various times the eggs were examined with a phase contrast microscope to determine the condition of cortical granules and female pronucleus.

Continuous exposure to high concentrations (10-20 µM) of A23187 was deterimental to the eggs, resulting in disintegration of the cytoplasm. Exposure to a lower concentration (3 µM), activated the eggs, the extent of activation being greater in Ca2+-Mg2+-free media (Table 2a) than in the complete medium (Table 2b). The rate of cortical granule breakdown and pronuclear development in Ca2+-Mg2+-free media was comparable with the rate in sperm-activated eggs8. The extent of cortical granule breakdown, however, was usually less in A23187-activated eggs than in those activated by sperm. Qualitative estimates showed 80-90% breakdown of granules in eggs activated in Ca<sup>2+</sup>-Mg<sup>2+</sup>-free media and a less complete breakdown in eggs activated in the complete medium. Activation of nuclear events were also slower in the complete medium. These differences may have resulted from decreased solubility of the ionophore in the presence of divalent cations.

Brief exposure to A23187 induced activation of the eggs. For example, when the eggs were exposed to  $3-10~\mu M$  A23187 in Ca<sup>2+</sup>-Mg<sup>2+</sup>-free BWW for 2 min then washed and left in Ca<sup>2+</sup>-Mg<sup>2+</sup>-free BWW, they all showed breakdown of the cortical granules within 30 min and well developed female pronuclei were formed in 2 h.

The activation by A23187 induced a zona reaction identical to that resulting from normal fertilisation. This is a change in the zona pellucida surrounding the egg, which prevents sperm penetration<sup>9, 10</sup>. Unfertilised cumulus-free eggs were rinsed with Ca2+-Mg2+-free BWW washed and left in Ca2+- $Mg^{2+}$ -free BWW, incubated for 2 min in 3-10  $\mu M$  A23187 in  $Ca^{2+}$ -Mg<sup>2+</sup>-free BWW for 30 min at  $37^{\circ}$ -38° C. The eggs were then transferred to a medium containing capacitated hamster sperm. The spermatozoa were incubated in a mixture of heat-inactivated rabbit serum and Tyrode solution (1:1) for 3-4 h to induce capacitation and the acrosome reaction before mixing with the eggs. The eggs were incubated with capacitated sperm at 37°-38° C for 2 h. No spermatozoa penetrated the zonae pellucidae of these eggs (45 eggs tested). Control eggs (38 tested) treated in the same way except for exposure to A23187 were all penetrated by spermatozoa.

Calcium has long been implicated to some extent in the activation of marine eggs. The usual procedure to induce some degree of parthenogenetic development was to substitute high concentrations of calcium for sodium in artificial seawaters<sup>11, 12</sup>. Also consistent with a Ca<sup>2+</sup> involvement was the observation that an increase in free cytosol calcium occurs in eggs which have just been fertilised<sup>13, 14</sup>. Previous results with the sea urchin indicate that activation can result from the release of intracellular stores of calcium induced by a calcium ionophore<sup>1</sup>. The Ca<sup>2+</sup>-ionophore can also activate a wide

Table 2 Activation of hamster eggs by A23187 (continuous exposure)

(a)	3	$\mu M$	A231	87	in	Ca2	+-Mg	2+	-free	BW	W/

% of egg nuclei exhibiting\*:

Time examined (min)	Cortical granules	Metaphase- II	Late anaphase-II to Early telophase-II	Mid-telophase- II	Early pronucleus	Well developed pronucleus	% of eggs degenerated
0 30 40 60 80 120 (120 min control) no A23187 treatme	++++ + + + + + + + +	100 0 0 0 0 0 0 0	0 100 0 0 0 0	0 0 100 0 0 0	0 0 100 14.3 0	0 0 0 0 78.5 73.5	0 0 0 7.2 26.5
0 30 40 60 120		100 100 - 80 - 53.8	37 in normal (Ca <sup>2+</sup> -M 0 0 20 23.2 0	g <sup>2+</sup> -containing) B 0 0 0 0 23.0 0	0 0 0 0 0 21.4	0 0 0 0 42.9	0 0 0 0 14.3

<sup>\* 30-40</sup> eggs in each group.

variety of species of eggs as reported here; and these activations of marine, amphibian and mammalian oocytes are also independent of external calcium. We now propose that release of intracellular ionic calcium may be the universal factor promoting activation of egg metabolism at fertilisation.

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## Concanavalin A-induced agglutination of Acanthamoeba

VIRULENT strains of Acanthamoeba produce cytopathic effects in mammalian tissue cultures<sup>1-3</sup>, and cause acute meningoencephalitis in laboratory animals after intranasal inoculation. In humans, Acanthamoeba seem to produce cerebral lesions in defence-weakened individuals<sup>4-7</sup>. We have begun studies of the cell surface of Acanthamoeba since virulent strains must make direct contact with a host cell before initiation of degenerative changes in mammalian tissue cultures2,3. The sensitivity of avirulent and virulent strains to concanavalin A (con A)-induced agglutination was investigated first as there was no evidence that carbohydrate-containing components were exposed at the cell surface of Acanthamoeba. Our results indicate that both avirulent and virulent Acanthamoeba were sensitive to con A-induced agglutination. In contrast to the results of studies of avirulent and virulent Entamoeba histolytica<sup>8</sup>, however, the avirulent strain was more sensitive to con A.

The avirulent Neff strain of A. castellanii and a virulent A-3 strain of A. culbertsoni<sup>10</sup> were cultured under constant axenic, suspension growth conditions as described previously11. The strains were removed from exponential cultures of approximately the same age, concentrated by centrifugation and washed three times with calciummagnesium-free-phosphate-buffered saline (CMF-PBS). Cells  $(5 \times 10^{5} \text{ ml}^{-1})$  were placed in Microtest tissue plates (Falcon) and, unless otherwise indicated, treated with the specified concentration of con A (crystallised three times; Miles-Yeda) with constant agitation at 22° C for 5 min. Agglutination was scored immediately on a scale of 0 to 4+ under an inverting microscope. Studies with ferritin-labelled con A (prepared as before12) were performed either at 4° C or 22° C, using solutions pre-equilibrated before addition of the cells. After incubation for 30 min, cells were maintained at 4° C or 22° C during three washes with CMF-PBS and preparation for electron microscopy.

Table 1 shows that under identical conditions, the avirulent strain of Acanthamoeba consistantly exhibited twice the sensitivity to con A-induced agglutination than that shown by the virulent strain. Simultaneous addition of 3.5 mM α-methyl-D-mannopyranoside abolished the agglutination of both strains. Nonspecific sugars such as lactose affected agglutination only at significantly higher concentrations (Table 1).

We further investigated whether agglutination could be affected by various treatments. Agglutination of both strains, tested at different concentrations of con A (avirulent: 0.5, 5 or 25 μg ml<sup>-1</sup>; virulent: 5, 25, or 50 μg ml<sup>-1</sup>), was unaffected after pretreatment for 15 min at 31° C with trypsin or pronase (0.0001 to 0.01 mg ml-1), neuraminidase (Vibrio cholera; 15 U ml-1; General Biochemicals) or ethylenediaminotetraacetic acid (0.1-1.0 mM). On the other hand, both strains pretreated for 15 min at 31° C with. sodium cyanide (16 mM), sodium arsinite (5 mM) or glutaraldehyde (0.25%) showed no agglutination after exposure to the above concentrations of con A. These results suggested that con A-mediated agglutination of Acanthamoeba might depend on free mobility of receptor sites in the membrane and also require ATP. Glutaraldehyde inhibition of con A-induced agglutination of transformed 3T3 fibroblasts has been explained similarly 13,14; however, ATP apparently is not necessary for con A-induced agglutination of these cells14.

As Fig. 1 shows, agglutination of both strains also was inhibited significantly at temperatures of 15° C or less. The temperature sensitivity further appeared to depend on the con A concentrations, and cells treated with con A at 5° C agglutinated rapidly when the temperature was raised to 22° C. Although inhibition of Acanthamoeba agglutination at low temperatures might be due to an alteration in the fluidity of the membrane14, we cannot yet exclude the possibility that the inhibition is due to some other cellular alteration, for example, inhibition of an essential enzyme(s). Since con A has been shown to bind at low tempera-

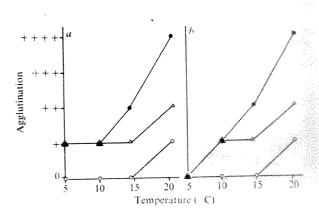


Fig. 1 Effect of temperature on con A-induced agglutination of avirulent (a) and virulent (b) Acanthamoeba. Concentrations of con A ( $\mu$ g ml<sup>-1</sup>) were (a):  $\bullet$ , 20;  $\triangle$ , 10;  $\circ$ , 1.5; (b):  $\bullet$ , 50;  $\triangle$ , 25;  $\bigcirc$ , 7.5.

Table 1 Con A-induced agglutination of avirulent and virulent strains of Acanthamoeba

Addition		ination†
CMF-PBS*	Avirulent 0	A STATE OF THE PARTY OF THE PAR
Con A‡ (µg ml <sup>-1</sup> )	U	0
2.5		0
	+	0
5.0	++	0
7.5	-	+
10.0	+++	-
15.0	++++	++
25.0	++++	++
50.0	++++	++++
Con A§ and α-methyl-D-mannopyranoside (3.5 mM)	0	0
(3.3 111141)	U	U
Con A§ and lactose (mM)		
5.0	++++	++++
10.0	++++	++++
25.0	++++	1111
50.0	++++	
100.0		1111

\* Cells were placed at a concentration of 5×105 cells ml-1 in

CMF-PBS and served as controls in all experiments. † 0, occasional clumps of three or less cells; 2+, ~50% of cells in clumps of 8 to 10 cells; 4+, >95% of cells in large clumps; -, not

‡ Con A was prepared in CMF-PBS.

 $\S$  The con A concentration in the competition experiments was 15  $\mu g$  ml<sup>-1</sup> or 50  $\mu g$  ml<sup>-1</sup> for the avirulent or virulent strains, respectively.

tures14,15, Acanthamoeba was treated with ferritin-labelled con A at 4° C or 22° C in an attempt to explain the greater agglutination of avirulent compared with virulent cells. In accordance with explanations for the similar sensitivity of transformed or trypsin-treated 3T3 fibroblasts compared with the normal fibroblast, the difference might be due to the amount of con A bound14,16, topographical distribution of the receptor sites17, and/or mobility of the receptor sites in the lateral plane of the membrane 13,14

Figure 2 shows high magnification micrographs of the cell surfaces of avirulent and virulent Acanthamoeba after exposure to ferritin conjugate at 4° C. The conjugate was

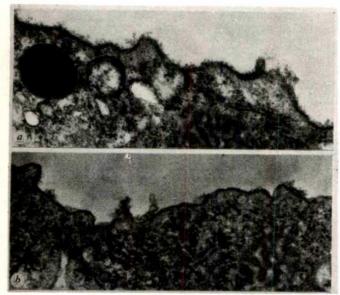


Fig. 2 Electron micrographs of thin sections of avirulent (a) or virulent (b) Acanthamoeba exposed to ferritin-labelled con A (80  $\mu$ g ml<sup>-1</sup>) at 4° C. Cells were placed at 5 × 10<sup>3</sup> cells ml<sup>-1</sup> in pre-equilibrated solutions of ferritin conjugate and incubated for 30 min. After several washes in CMF-PBS, cells were fixed with glutaraldehyde and osmium tetroxide, embedded in Araldite, and the sections stained with uranyl acetate and lead citrate  $(a, \times 36,480; b, \times 43,920)$ .

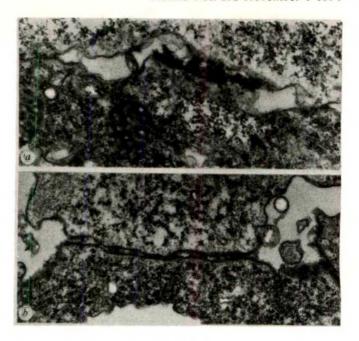


Fig. 3 Electron micrographs of thin sections of avirulent (a) or virulent (b) Acanthamoeba exposed to ferritin-labelled con A (40  $\mu$ g ml<sup>-1</sup>) at 22° C. After incubation in the conjugate for 30 min, samples of cells were processed as described in Fig. 2. The micrograph shown in (b) was typical of the contact regions of both agglutinated virulent and avirulent amoebae. The micrograph in (a) illustrates the electron-dense, amorphous material with associated ferritinlabelled con A which was found occasionally in contact regions of agglutinated avirulent amoebae (a, ×29,280;  $b, \times 19,200$ ).

distributed randomly with only an occasional cluster of the ferritin label at the surface of the avirulent amoebae (Fig. 2a). In contrast, the surface of the virulent cells appeared almost devoid of associated ferritin-labelled con A. High magnification revealed, however, that the conjugate was localised in discrete clusters on the virulent amoebae surface (Fig. 2b). In both avirulent and virulent cells, little or no label was found within the cytoplasm but, when present, it was enclosed in small membrane-bound vacuoles (Fig. 2a). Sections of control preparations, in which cells were exposed only to ferritin, showed no nonspecific binding of ferritin molecules.

Figure 3 shows electron micrographs of avirulent and virulent Acanthamoeba exposed to the ferritin-labelled con A at 22° C and illustrates regions of contact between agglutinated cells in the samples. High concentrations of the conjugate were observed routinely on membrane areas immediately adjacent to and at contact points of both agglutinated avirulent and virulent amoebae. Areas more distal to the contact regions were relatively free of conjugate. Figure 3b, although showing agglutinated virulent amoebae, is typical of the appearance of most contact regions between avirulent cells. Additionally, however, contact regions of avirulent amoebae occasionally contained large amounts of electron dense, amorphous material with associated ferritinlabelled con A (Fig. 3a). Although not illustrated, the ferritin conjugate was clustered on the surfaces of nonagglutinated, single cells in samples of both avirulent and virulent cells treated with the conjugate at 22° C.

In view of the uniform distribution of ferritin conjugate at the surface of avirulent amoebae exposed at 4° C, the greater agglutination of this strain cannot be attributed to the presence of pre-clustered con A receptor sites, which may account for the greater sensitivity of the transformed 3T3 fibroblast to con A17. Instead, the greater sensitivity of the avirulent strain probably results because more con A receptor sites (mannose-like residues) are exposed at the surface than on the virulent strain. A greater mobility of the con A receptor sites in the membrane of the avirulent strain may contribute partially to the greater agglutination; however, since the virulent strain appeared capable of clustering con A receptor sites, as evidenced by the large aggregates associated with contact points between agglutinated cells in samples exposed to ferritin conjugate at 22° C, the difference in the membrane fluidity of the strains may be minimal.

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## Dissociation of increased hexose transport from initiation of fibroblast proliferation

ALTERATIONS in the permeability of the surface membrane might play a key role in the control of DNA synthesis and cell division<sup>1-3</sup>. Evidence that changes in hexose transport might participate in growth control comes from reports that uptake of certain hexoses (1) increases after transformation by tumour viruses4-6, and correlates with the expression of the transformed phenotype<sup>7</sup>; (2) decreases when untransformed fibroblasts reach a density-inhibited monolayer<sup>5,8</sup>, and (3) rapidly increases after initiating cell division with serum or other agents<sup>8,9</sup>. We now report, however, that increased hexose transport and initiation of proliferation can be uncoupled in density-inhibited 3T3 mouse fibroblasts. Levels of cortisol which initiate DNA synthesis and division of these cells18,111 actually decrease hexose transport. In addition, some serum components increase hexose transport without initiating proliferation.

Mycoplasma-free 3T3 Swiss mouse fibroblasts (clone 42) were grown as previously described12. Medium components were purchased from Gibco. Density-inhibited cultures were prepared by plating 30 or 50 mm Nunc plastic tissue culture dishes with  $1.7 \times 10^4$  cells cm<sup>-2</sup> in 2 ml (for 30

Table 1 Stimulation of DNA synthesis and cell division by cortisol and serum

A delition	cpm <sup>3</sup> H-thymidine	cells × 10 <sup>-4</sup>
Addition  None 3 × 10 <sup>-6</sup> M Cortisol 2.5% Serum 5.0% Serum 7.0% Serum	10 <sup>5</sup> cells 235 1,267 889 1,372 1,622	3.6 4.9 4.0 4.5 5.2

Cells were grown to confluency and cortisol or fresh serum was added to cultures. DNA synthesis and cell number were determined at 24 and 72 h respectively after these additions as previously described 10.

mm dishes) or 4.5 ml (for 50 mm dishes) of culture medium containing 10% calf serum. The cells grew to a final saturation density of  $3.5 \times 10^4$  to  $4.2 \times 10^4$  cells cm<sup>-2</sup> over 3 d. Hexose uptake was measured using the nonmetabolisable hexose analogues 3-O-methyl-p-glucose and 2-deoxy-D-glucose. Both of these sugars are transported into cells by facilitated diffusion. Kinetic studies have indicated that they are transported by the same system that transports glucose.

To determine the extent of correlation of initiation of proliferation and increased hexose transport, we added fresh serum (5.0%) or the glucocorticoid steroid hormone, cortisol (3.0×10<sup>-6</sup> M) and measured DNA synthesis, cell number and uptake of hexose analogues. These additions caused similar increases in the rate of DNA synthesis at 24 h and cell number at 72 h (Table 1), indicating that under these conditions measuring DNA synthesis was an \* accurate measure of proliferation. As Fig. 1 shows, however, initiation of DNA synthesis in density-inhibited 3T3

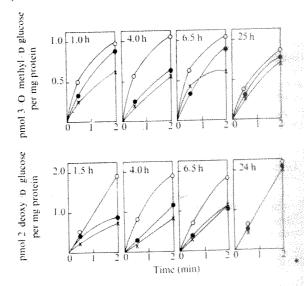


Fig. 1 Time course of 3-O-methyl-p-glucose and 2-deoxy-pglucose uptake by control density-inhibited 3T3 cultures (a) and parallel cultures treated with 5% serum (), or 3 µM  $cortisol(\times)$ . Times in hours refer to times after serum or cortisol treatment. Hexose uptake was measured by removing the growth medium, rinsing the cells once with PBS at 37° C and adding <sup>3</sup>H-3-O-methyl-D-glucose (2 µCi; 3.7 Ci mmol<sup>-1</sup>) or <sup>3</sup>H-2-deoxy-D-glucose (2 µCi; 7.2 Ci mmol<sup>-1</sup>) in 2.0 ml PBS. Cultures were incubated at 37° C for the times indicated on the abcissa. They were then immediately rinsed four times with PBS at 4° C. Less than 5% of the intracellular radioactivity was removed by this rinsing procedure. Further rinsing did not remove significant amounts of radioactivity from the cells. Acid-soluble radioactivity was extracted from the cells for one hour with 10% trichloroacetic acid (TCA) at 4° C, and measured in a liquid scintillation counter. Uptake measurements were corrected for radioactivity nonspecifically associated with the cells which was consistently less than 10% of the uptake value. Nonspecific association was determined by adding labelled hexose in PBS to cells at 4° C. This was immediately aspirated, and cultures were processed as described above for uptake measurements.

Table 2 Relative stimulation of DNA synthesis and hexose uptake by different lots of commercial serum

			wommin	ciai sciaini		
A	ddition	Thymidine inco		2-deoxy-D-glucose uptake		
		c.p.m. × 10 <sup>-4</sup>	% control	pmol	% control	
		per mg protein		per mg protein		
	one	0.11	100	0.90	100	
C	733308	1.05	950	1.42	159	
A	9219E	2.66	2420	1.14	128	
R	2034R	2.12	1930	1.07	119	
R	832101	2.51	2280	1.02	114	
R	636017	2.14	1950	1.01	113	
	20720	2.47	2240	0.98	110	

After growth to confluency, 5% fresh calf serum was added to cultures. Uptake of ( $^{3}$ H) 2-deoxy-D-glucose (0.5  $\mu$ Ci ml $^{-1}$ ; 7.2 Ci mmol $^{-1}$ ) was measured during a 1-min pulse 4 h after the addition of serum as described in the legend to Fig. 1. DNA synthesis was measured at 24 h $^{10}$ . Protein was measured as previously described $^{19}$ .

fibroblasts was not always accompanied by increased hexose uptake. Addition of fresh serum to a final concentration of 5% brought about an increase in uptake of both 3-O-methyl-D-glucose and 2-deoxy-D-glucose by 1 h. The increase was maximal by 4-6 h and declined to values characteristic of density-inhibited cells by 24 h. This result confirms earlier findings. In contrast to serum, cortisol inhibits hexose uptake by certain cells 13,14. Indeed, Fig. 1 shows that  $3 \times 10^{-6}$  M cortisol, a concentration which initiated DNA synthesis and division similar to 5% serum (Table 1), actually brought about a small decrease in transport of both hexose analogues by density-inhibited 3T3 fibroblasts. Figure 2 shows that this transport decrease occurred with several cortisol concentrations which initiated DNA synthesis. Thus, an increase in hexose transport is not necessary for subsequent proliferation.

While 2-deoxy-D-glucose has been commonly used to measure glucose transport, it has the disadvantage of being phosphorylated. Thus, the intracellular radioactivity represents the sum of the phosphorylated and unphosphorylated forms of this sugar, and altered rates of transport might actually reflect altered phosphorylating activity. Since we obtained similar results with 3-O-methyl-D-glucose, which is not phosphorylated, the observed alterations appear to be a result of transport changes rather than phosphorylation.

To probe further the relationship between initiation of proliferation and increased hexose transport, we added various levels of fresh serum (0.025% to 25%) to density-inhibited 3T3 fibroblasts and measured 3-O-methyl-pglucose uptake, DNA synthesis and cell number. The dose-response curves in Fig. 3 show that fresh serum added to 0.5% caused a maximal increase in hexose transport but no• significant increase in DNA synthesis or cell number.

As Fig. 3 shows, higher concentrations of serum brought about increases in DNA synthesis and cell number which were roughly proportional to serum concentration. These higher concentrations caused no additional increase in hexose uptake. Thus, an early increase in hexose uptake is not sufficient by itself to initiate proliferation of density-inhibited 3T3 fibroblasts.

Additional experiments indicated that serum might contain multiple factors which affect growth and hexose uptake and that the relative activities of these factors might vary in different lots of serum. For example, dose-response studies, like those shown in Fig. 3, revealed for some lots of serum parallel increases in hexose uptake, DNA synthesis and cell number (data not shown). In addition, the relative stimulation of DNA synthesis and hexose uptake varied with different commercial serum lots (Table 2). In fact, the lot which produced the greatest stimulation of hexose uptake had the smallest effect on DNA synthesis. Moreover, various pure or partially purified fractions of serum had differential effects on hexose uptake and DNA synthesis (Table 3). Addition of fetuin (0.5 mg ml<sup>-1</sup>) produced a significant stimulation of hexose uptake but had no effect on DNA synthesis. As previously noted, cortisol stimulated DNA synthesis but not hexose uptake. Addition of insulin produced a graded increase in both responses (Table 3).

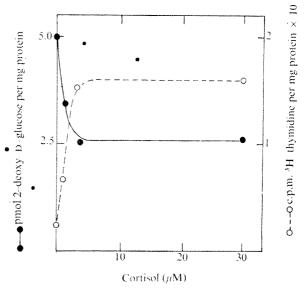


Fig. 2 Effect of cortisol concentration on hexose uptake and DNA synthesis by density-inhibited 3T3 cells. Uptake of 2-deoxy-D-glucose (●) was measured 4 h after cortisol addition as described in the legend to Fig. 1. DNA synthesis (○) was measured 24 h after cortisol addition as previously described¹¹⁰.

Table 3 Stimulation of DNA synthesis and hexose uptake by fetuin, cortisol, and insulin

	Addition	Thymidine inco	orporation	3-O-methyl-D-glucose uptake		
_		c.p.m. × 10 <sup>-4</sup> per mg protein	% control	pmol per mg protein	% control	
1	None	$1.89 \pm 0.15*$	100	$0.98 \pm 0.19$	100	
	0.5 mg ml <sup>-1</sup> fetuin	$1.96 \pm 0.15*$	103	$1.61 \pm 0.18$	166	
	10% serum	4.06±0.19*	214	$2.56 \pm 0.33$	264	
II		1.10±0.22*	100	$1.48 \pm 0.06$	100	
	3×10 <sup>-6</sup> M cortisol	$2.38 \pm 0.07$ *	216	$1.43 \pm 0.11$	97	
Ш	None 0.5 ng insulin per ml 200 ng insulin per ml	$\begin{array}{c} 0.25 \pm 0.03 \dagger \\ 0.43 \pm 0.08 \dagger \\ 0.80 \pm 0.13 \end{array}$	100 172 320	$\begin{array}{c} 1.50 \pm 0.20 \\ 1.82 \pm 0.04 \\ 2.16 \pm 0.04 \end{array}$	100 121 144	

Fetuin, cortisol or insulin were added to confluent cultures and uptake of 3-O-methyl-D-glucose (0.5 μCi ml<sup>-1</sup>; 3.7 Ci mmol<sup>-1</sup>) was measured during a 1-min pulse 4 h later as described in the legend to Fig. 1. DNA synthesis was measured as previously described<sup>10</sup>.

\* Cultures labelled for 15 min 24 h after additions.

<sup>†</sup> Cultures labelled for 6 h from 21 to 27 h after additions.

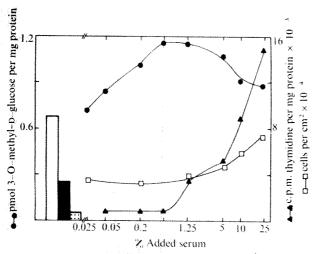


Fig. 3 Effect of concentration of added fresh serum on 3-Omethyl-D glucose uptake (●), DNA synthesis (▲) and cell number ([]). 3T3 cells were grown to confluency in medium containing 10% calf serum. Fresh serum was then added to the concentrations indicated on the abcissa. Hexose uptake was measured as described in the legend to Fig. 1 during 1 min incubation 4 h after addition of fresh serum. DNA synthesis was measured at 24 h (ref. 10) and cell number was monitored at 72 h by counting in a haemocytometer. Vertical bars show control values for density-inhibited cultures: plain bar, hexose uptake; solid bar, cell number; stippled bar, DNA synthesis.

These results demonstrate that increased hexose uptake is neither a necessary nor sufficient event for proliferation of density-inhibited 3T3 cells. •Cortisol stimulates DNA synthesis and cell division in the absence of an early increase in hexose transport, and addition of fetuin or low concentrations of serum produce increased hexose uptake without subsequent proliferation. The conclusion that increased hexose uptake and proliferation may be unlinked is consistent with the findings of Gazdar et al. that treatment of mouse sarcoma virus-transformed BALB/c 3T3 cells with dibutyryl cyclic AMP and theophylline sharply inhibited cell growth yet stimulated glucose uptake15. In addition, Rubin and Fodge have suggested that 2-deoxy-Dglucose uptake merely reflects the activity of the glycolytic pathway which is activated after stimulation proliferation16.

Early transport increases for phosphate and uridine have also been implicated in the initiation by fresh serum of proliferation of density-inhibited fibroblasts17,18. In addition, Holley has shown that 3T3 cells can be arrested in G<sub>1</sub> (or G<sub>0</sub>) by limiting phosphate in the medium, and that adding back phosphate leads to reinitiation of DNA synthesis3. De Asua et al. showed that addition of phosphate to quiescent 3T3 cells brought about a decrease in intracellular cyclic AMP levels and was required for full activation of uridine transport by serum18. They suggest that the increase in phosphate transport is a primary event in the reinitiation of growth. Cunningham and Pardee, however, showed that certain serum fractions, obtained by gel filtration on Sephadex G-200, initiated DNA synthesis in density-inhibited 3T3 fibroblasts but did not produce an increase in phosphate uptake. Moreover, fractions which increased phosphate uptake did not initiate DNA synthesis<sup>17</sup>. Thus, the early increase in phosphate transport is neither necessary nor sufficient for fibroblast proliferation brought about by fresh serum. We are probing further the possible involvement of changes in phosphate uptake in growth control processes.

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## Dual mechanism for the action of cholesterol on membrane permeability

CHOLESTEROL, which is a widespread component of the plasma membrane, has a substantial influence on the permeability properties of lipid bilayer1-3 and cell membranes4. Although the effects of cholesterol on membrane structure are increasingly well understood<sup>5</sup> the mechanisms by which cholesterol modulates membrane permeability are obscure, mainly because it is difficult to deduce permeability properties from the knowledge of membrane structure alone.

We describe here quantitative determination of the influence of cholesterol on those variables of membrane structureelectric potential, chemical partition and rate of ionic transferwhich directly govern ionic permeability. The method useds compares the ionic conductances induced by positively charged lipophilic species to those induced by negatively charged lipophilic species. Such probing of monoolein membranes shows that although cholesterol somewhat decreases the rate of ionic transfer in these membranes, its predominant effect on ionic permeability arises by increasing the electric potential within the membrane interior, presumably by altering the strength and orientation of dipolar groups present at the membrane surface, since charged groups are absent.

The electrical conductance induced in Mueller and Rudin type7 bilayers by the lipophilic anions tetraphenylborate (TFB-) and carbonylcyanide m-chlorophenylhydrazone (CCCP-) as well as the lipophilic cations tetraphenyl phosphonium (TFP+) and 3,3'-dipropyloxadicarbocyanine iodidediO-C<sub>5</sub>-(3) (CC<sub>5</sub>+)8 was characterised as a function of the

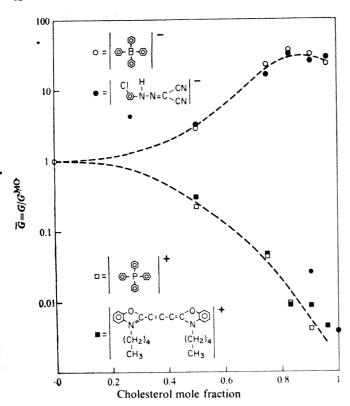


Fig. 1 Opposite effect of cholesterol on relative conductance of lipophilic cations and anions. Ordinate: relative conductance on a logarithmic scale. Abscissa: mole-fraction of cholesterol in the membrane-forming monoolein cholesterol solution. The total lipid concentration was 25 mM in decane. ○, TFB⁻; ●, CCCP⁻; □, TFP⁺; ■, CC₅⁺. For TFB⁻ equilibrium values of the relative conductance were obtained by rapid conductance measurements⁰. The supporting electrolyte was 1 M NaCl. The solutions were buffered only for CCCP, at pH 6.0 with phosphate.

cholesterol-monoolein mole-fraction in the membrane-forming lipids (25 mM in decane) using previously described techniques<sup>1</sup>. The measurements were made on membranes separating aqueous solutions of identical composition, in the limit of small applied d.c. potentials ( $\pm$  10 mV) for which conductances are voltage-independent. Membrane conductances were independent of the type (NaCl or KCl) or ionic strength ( $10^{-2}$  M to 1 M) of the supporting electrolyte, confirming the expected absence of charged polar head groups in the monoolein-cholesterol bilayers<sup>6</sup>. For each of the lipophilic ions, the relative conductance,  $\bar{G} = G/G^{MO}$ , (defined as the ratio between the conductance of a cholesterol-rich membrane, G, and that of a pure monoolein membrane,  $G^{MO}$ , formed in a solution of the same composition) was found to be independent of the pH and of the lipophilic ion concentration over a wide range.

Figure 1 plots relative conductances for CCCP<sup>-</sup>, TFB<sup>-</sup>, TFP<sup>+</sup>, and CC5+ as a function of increasing cholesterol-monoolein mole-fraction in the membrane-forming lipids. The most notable feature of Fig. 1 is the opposite effect of increasing cholesterol mole-fraction on the conductance of lipophilic anions and cations: the conductance for anions (CCCP- and TFB-) is increased about 30-fold while the conductance for cations (TFP+ and CC<sub>5</sub>+) is decreased about 100-fold. Moreover, an increase in the cholesterol mole-fraction has the same effect, within the experimental accuracy, on ions of the same charge in spite of the differences in the chemical structure and molecular shape and size of these ions (compare CCCP- with TFB- or TFP+ with CC<sub>5</sub>+). In contrast, an increase in the cholesterol mole-fraction has an opposite effect on ions with opposite charges in spite of the identical structure of these ions (compare TFB- with TFP+). Evidently it is the charge, and not the detailed chemical properties or molecular size, that determines the way in which conductance is altered by cholesterol. The lipophilic ions appear to 'sense' an increasingly positive electrostatic potential in membranes of increasing cholesterol content. Furthermore, the opposite changes seen for cations and anions in Fig. 1 imply that the membrane conductance is influenced more by this potential than by changes in membrane fluidity, which should decrease the conductance for cations and anions.

To analyse quantitatively the data of Fig. 1, consider for a lipophilic ion of valance z, the theoretical expression of the relative conductance,  $\overline{G}$ , written as a function of  $\Delta \Psi_0 = \Psi_0 - \Psi_0^{MO}$ ,  $\overline{k} = k/k^{MO}$  and  $\Delta \mu^0 = \mu^0 - \mu^{0MO}$  which respectively denote the change in the electrostatic potential  $(\Psi_0)$ , in the rate of ionic transfer (k) and in the chemical free energy of partition  $(\mu^0)$  relative to a monoolein membrane (superscript MO)<sup>6.9</sup>.

$$\overline{G} = G/G^{MO} = [\exp(-zF\Delta\psi_0/RT)][(\overline{k}\exp(\Delta\mu^0/RT)]]$$

The two factors on the right-hand side of this equation have a simple physical meaning. The first factor describes the effect of the surface potential on membrane conductance. The second factor, which is the intrinsic conductance change that would be measured if there were no surface potential change, describes the effect of diffusion and chemical partition on membrane conductance. These two factors can be evaluated separately from the relative conductance for cationic  $(\overline{G}^+)$  and anionic  $(\overline{G}^-)$  species if the second factor depends on lipid composition in the same way for cationic and anionic species.

$$\exp(F\Delta\psi_0/RT) = (\overline{G}^+/\overline{G}^-)^{-1/2}; \overline{k} \exp(\Delta\mu^0/RT) = (\overline{G}^+/\overline{G}^-)^{1/2}$$

The data of Fig. 1 show that, within experimental accuracy, a single relative conductance,  $\overline{G}^+$  and  $\overline{G}^-$  respectively, describes the effect of cholesterol on different cationic and anionic species. This, together with the fact that TFB<sup>-</sup> and TFP<sup>+</sup> have the same molecular structure, implies that the condition for separating surface potential and intrinsic conductance is satisfied for the four ions of Fig. 1.

Figure 2 plots calculated values of relative surface potential (upper part) and intrinsic conductance change (lower part) as a function of cholesterol-monoolein mole fraction. The upper part of Fig. 2 shows that in accordance with intuitive expectation the electrostatic potential increases in the membrane interior as the cholesterol-monoolein mole-fraction is increased. This surface potential increase is large; it has a maximal value of 110 mV for predominantly cholesterol bilayers compared with pure monoolein bilayers and it accounts for most of the observed permeability changes. Since the surface-potential is independent of salt concentration, and furthermore, since the lipid has no ionisable polar head groups (that is, there are only —OH

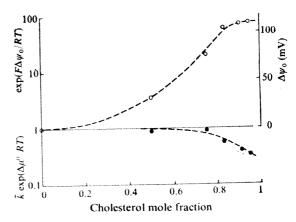


Fig. 2 Relative contributions of dipolar surface potential and intrinsic conductance changes to the effect of cholesterol on the membrane conductance induced by the lipophilic ions CCCP<sup>-</sup>, TFB<sup>-</sup>, TFP<sup>+</sup> CC<sub>5</sub><sup>+</sup> in monoolein membranes. The experimental data of Fig. 1 were analysed according to the procedure outlined in the text. Averaged values of the relative conductance for CCCP<sup>-</sup> and TFB<sup>-</sup> were used as  $\overline{G}$ <sup>-</sup>; averaged values of the relative conductance for TFP<sup>+</sup> and CC<sub>5</sub><sup>+</sup> were used as  $\overline{G}$ <sup>+</sup>.

groups, which are neutral at the pH of the experiments), the change in the surface potential must have its origin in changes in the orientation, strength and packing density of molecular dipoles at the membrane surface.

The existence of such dipolar surface-potential changes is not unreasonable10.11. Indeed, about 90 mV difference of the correct polarity is found between compensation potentials of cholesterol (near 390 mV)12 and monoolein (near 300 mV)10 monolayers spread at the air-water interface. Since a monolayer can be viewed as one half of a bilayer, the monolayer data lend independent support to the surface potentials deduced here solely on the basis of bilayer measurements, although care must be taken in making such comparisons since compensation potentials are steeply dependent on the extent to which the monolayer is compressed.

The lower part of Fig. 2 shows that cholesterol has a significant but small effect on intrinsic conductance compared with its main effect, caused by changes in surface potential (compare the left-hand scales on the upper and lower part of Fig. 2). Current-voltage measurements (that is, an exp(FV/2RT) type dependence of the current on voltage) indicate that the peak of the energy barrier13, and therefore the rate-limiting step for the permeation of CCCP-, TFB-, TFP+, and CC<sub>5</sub>+, is located in the middle of the membrane. The small effect of cholesterol on intrinsic conductance shows, therefore, that the fluidity of the middle of the membrane is not much altered by cholesterol. This supports Rothman and Engelman's conclusion that cholesterol decreases fluidity only near the membrane surface and not in the membrane interior.

It should be emphasised that the influence of cholesterol on intrinsic conductance is not always small. Preliminary experiments indicate that cholesterol decreases substantially (that is, about 100-fold) intrinsic conductance for sodium complexes of the neutral carriers, valinomycin, nonactin and triactin. Current-voltage measurements (that is, an exp(FV/2.8RT) type dependence of the current on voltage) indicate that the energy barrier is trapezoidal18 and therefore the rate-limiting step for the permeation of these complexes is located nearer to the membrane surface, where cholesterol is expected to have its maximal effect on fluidity.

Probing of cholesterol-containing monoolein bilayers reveals that cholesterol modulates membrane permeability by two distinct mechanisms: by altering (1) the electrical potential difference across the membrane-solution interface, thereby changing the partition of charged species into the membrane, and (2) the fluidity of the membrane interior, thereby changing the rate of ionic transfer and the chemical partition of the solute in the membrane. The first mechanism is expected to act in the same way on the permeability of changed species of widely different molecular structure. In contrast, the second mechanism is expected to act differently on the permeability of different ionic species depending on the structural details of the membrane and of the ion. For CCCP-, TFB-, TFP+ and CC5+ the second mechanism has only a small influence. For typical ion carriers, such as valinomycin, nonactin and triactin, its influence appears to be more prominent.

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## Collagenase activity in experimental hepatic fibrosis

THE collagen fibres newly formed in experimental hepatic fibrosis and pre-cirrhosis are resorbed when the noxic stimuli have been removed1. In investigating the mechanism of genesis and resorption of collagen fibres in experimental hepatic fibrosis2,3 we have found using electron microscopy that the collagen fibres are degraded extracellularly and partly enguited by Kupffer cells and/or macrophages<sup>4,5</sup>. Within these cells digestion of collagen fibrils or degraded collagen fibrils may occur in lysosomes6.

Native collagen is resistant to all known proteolytic enzymes except collagenase, which is operative at neutral pH under physiological conditions. The first animal collagenase was isolated7.8 from the culture fluid of tadpole skin. Subsequently, collagenase was found in the culture fluid of many kinds of tissues, such as skin, postpartum rat uterus, bone, human gingiva, cornea and synovium, and in the synovial fluid from patients with rheumatoid arthritis. Collagenase has not yet been definitively observed in the liver of either animals or humans10. Here we report the presence of collagenase in rat liver and increased collagenase activity in experimental hepatic fibrosis induced by carbon tetrachloride.

Fifty female Wistar strain rats, with an average weight of 150 g, received subcutaneous injection of 0.3 ml of 50% CCI4 in olive oil per 100 g body weight, twice a week. Ten control rats were not treated. Nine to twelve rats were killed at 3, 6 and 20 weeks after the initial administration of CCl<sub>4</sub>. Diced specimens (2×2 mm) of rat liver were removed under sterile conditions and transferred immediately to 105 mm Petri dishes with sterile mammalian Tyrode's solution to which had been added 150 U penicillin ml<sup>-1</sup> and 140 µg streptomycin ml<sup>-1</sup>. They were washed repeatedly in mammalian Tyrode's solution for 24 h until they could be implanted on collagen substrate.

Salt-extracted collagen was prepared according to the method of Kang et al.11. The skin from young, growing Wistar strain rats was used as the source of collagen. The purity of saltextracted collagen was confirmed by disc electrophoresis and analysis of amino acid composition (manuscript in preparation). The assay method was similar in principle to that devised by Gross and Lapiere'. Two hundred and fifty microlitres of 0.1% collagen in cold mammalian Tyrode's medium was poured into microdishes 12 mm in diameter and 3 mm deep, and incubated at 37°C for at least 3 h. The reconstituted collagen gels were formed and were not digested by 50 µg of either trypsin, papain or pepsin (manuscript in preparation). Diced specimens of rat liver were placed on the rigid opalescent gel and incubated at 37° C for 72 h with mammalian Tyrode's solution in the outer ring (21 mm internal diameter and 5 mm deep) in a moist chamber containing 90% O<sub>2</sub> and 10% CO<sub>2</sub>. The lysis area around the explant was observed daily and the sterility of the culture was frequently confirmed.

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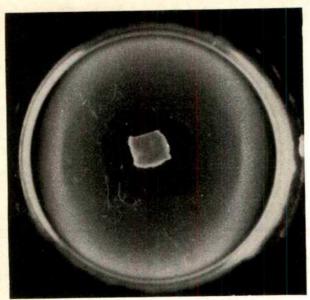


Fig. 1 Culture of rat liver treated with CCl4 for 6 weeks showing area of lysis around explant (magnification ×5.8).

In the ten non-treated rats, two livers demonstrated weak activity, five livers minimal activity and another three livers did not show appreciable lysis. Thus untreated rat livers have weak collagenolytic activity.

Microscopically the rat livers treated with CCl4 for 3 weeks showed slight to moderate periportal fibrosis. Six weeks after the initial administration of CCl4, fibrous septa were formed connecting portal tracts with the central canal containing a tributary of the hepatic vein. After 20 weeks, the rat liver showed the typical features of cirrhosis including regenerative nodules. Collagenolytic activity increased slightly in the initial stage of hepatic fibrosis and markedly in the extensive fibrosis. Figure 1 illustrates lysis of the collagen substrate surrounding an explant of rat liver treated with CCl4 administration for 6 weeks. The collagenolytic activity in the irreversible cirrhotic stage was poor compared with the activity in the rat liver treated with CCl4 for 6 weeks.

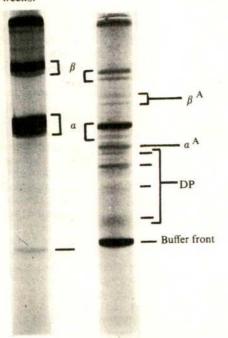


Fig. 2 Acrylamide gel electrophoresis patterns of products from incubations of collagen with rat liver treated with CCI<sub>4</sub> for 6 weeks (right) and without explant (left);  $\alpha$ , monomer;  $\beta$ , dimers;  $\alpha^{A}$ ,  $\beta^{A}$ , degradation products of  $\alpha$  and  $\beta$  respectively; DP, degradation products of lower molecular weight.

Acrylamide gel electrophoresis patterns of products from incubations of the collagen gel with rat liver treated with CCl<sub>4</sub> for 6 weeks are shown in Fig. 2. The control pattern consists of monomers (α), dimers (β) and higher molecular weight species. Incubation of collagen with the pre-cirrhotic rat liver at 35° C resulted in discrete products of α and β: α<sup>A</sup> and β<sup>A</sup> and degradative products of lower molecular weight which are similar to those seen with other animal collagenases12,13. Repeatedly frozen and thawed rat liver tissue failed to induce visible lysis while living tissue from the same rat caused appreciable lysis; collagenolytic activity was inhibited by addition of sodium EDTA to a final concentration of 0.01 M (manuscript in preparation). Neither aerobic nor anaerobic bacterial contamination was detected in gels where lysis was seen.

Thus the increased collagenolytic activity observed in experimental hepatic fibrosis must be due to collagenase. The proliferation of collagen fibres must be because collagen is synthesised more rapidly than it is resorbed. The discontinuation of toxic stimuli presumably results in increased collagenolytic activity to resorb the excessive collagen fibres. The mechanism of hepatic fibrogenesis in human beings may be more complicated; we shall investigate it from the viewpoint of collagen metabolism in the liver.

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## Glutamate metabolism in the frog retina

THE existence of a compartmented metabolism of glutamate in cerebral cortex is well established1.2. When, for example, glutamate, acetate or y-aminobutyric acid (GA) are metabolised by the tissue the specific activity of the glutamine formed exceeds that of the total tissue glutamate pool. As the immediate precursor of glutamine is glutamate, the implication must be that there is more than one pool of the latter. A model consisting of a minimum of three compartments seems most compatible with available data and compartment 1, from which glutamine is rapidly formed, is thought to be located in glial cells1.2.

Why metabolism within the central nervous system is organised in this way is not certain but it has been proposed2 that it may relate to the role of GABA as an inhibitory neurotransmitter-the suggestion being that GABA, when released by neurones, is taken up by the glia, replacement carbon atoms then being returned to the neurones in the form of glutamine.

There is evidence that GABA may function as a neurotransmitter in the vertebrate retina<sup>3,4</sup>. Wide species differences exist however<sup>4</sup>. In particular, autoradiography has shown that, in mammalian retinae, exogenously applied GABA is accumulated into glial cells<sup>5-7</sup>; in the rabbit retina amacrine cells are also labelled<sup>7-9</sup>. In contrast, in lower order vertebrates such as goldfish, frog, pigeon and chicken, GABA goes solely into neurones<sup>10-12</sup>. There are insufficient data from which to evaluate fully the role of GABA in mammalian and other retinae but the above results imply fundamental differences in the way that the two groups handle the amino acid.

More than one pool of glutamate is present in rat retina<sup>13</sup> and, as in brain, glutamate, aspartate, acetate, GABA, leucine and bicarbonate are all effective precursors of glutamine by way of a 'small' glutamate compartment. Because of the species differences in GABA uptake into retinal tissue and the postulated connection between glutamate compartmentation and the functional organisation of GABA, we thought it of interest to know how glutamate is metabolised in the frog retina. We have therefore investigated the metabolism of labelled acetate, glutamate and glucose in this tissue and have identified the cells accumulating <sup>3</sup>H-glutamic acid by light microscope autoradiography<sup>6</sup>.

All frogs (Rana temporaria) were dark adapted overnight to free the neural retina from extensive contact with the pigment epithelium. The retinae were then dissected out<sup>12</sup> under normal room lighting and incubated in 20 ml specimen bottles. At the end of the incubation period the labelled amino acids were extracted from the tissue and processed by•a modification of the method described by Starr<sup>13</sup>. The concentrations of the amino acids present in the retina, after control incubations in the presence of unlabelled substrate, were determined using double label dansylation<sup>14,15</sup>.

The results are detailed in Table 1. With glutamate or acetate as substrate, the specific activity (RSA) of glutamine relative to glutamate, after 30 min in vitro incubation, was greater than unity. In contrast, when glucose was the substrate, a normal precursor/product relationship was observed and the RSA was less than one. Thus a compartmentation of glutamate metabolism, qualitatively similar to that in the rat retina<sup>13</sup>, is present in the frog retina also. In addition the results imply that, as in other areas of the CNS, glutamate produced from glucose passes into the glutamate pool(s) from which glutamine is formed.

Table 1 Formation of aspartate, glutamate, glutamine and GABA from labelled precursors

Amino	C-glucose		1-14 C-acetate		U-14 C-glutamate		nmol per retina	
acid	SA	RSA	SA	RSA	SA	RSA	$\pm$ s.e.m. $16.6\pm2.1$ $40.4\pm0.7$ $23.7\pm1.0$ $28.6\pm0.6$	
Aspartate	58.3	0.28	67.6	0.29	369	0.13		
Glutamate	205	1.0	231	1.0	2757	1.0		
Glutamine	135	0.66	372	1.61	8952	3.25		
GABA	159	0.77	94	0.40	495	0.18		

Retinae were preincubated for 15 min at 25° C in 2 ml oxygenated bicarbonate Ringer<sup>13</sup> containing 50 mg % glucose and then incubated for 30 min in the presence of p-U-<sup>13</sup>C-glucose (2.5 µCi ml<sup>-1</sup>; 268 mCi mmol<sup>-1</sup>), 1-<sup>14</sup>C-sodium acetate (6.7 µCi ml<sup>-1</sup>; 56 mCi mmol<sup>-1</sup>) or L-U-<sup>13</sup>C-glutamic acid (2 µCi ml<sup>-1</sup>; 260 mCi mmol<sup>-1</sup>). After washing, free amino acids were isolated from the retinae and the extracts were evaporated to dryness, taken up in 100 µl water and 30 µl taken for separation on Whatman No. 1 chromatography sheets (descending)<sup>13</sup>. The solvent was saturated aqueous phenol. The amino acids were separately eluted with 1.0 ml water and the radioactivity estimated by liquid scintillation counting<sup>12</sup>. Standard quantities of glutamate, aspartate, glutamine and GABA, added to retinal homogenates, were processed and recoveries of 67-95% obtained. Experimental values were corrected accordingly. Endogenous amino acids were estimated by double label dansylation as previously described<sup>15</sup>.

SA denotes d.p.m. per µmol amino acid × 10<sup>-3</sup> and RSA the specific activity relative to glutamate.

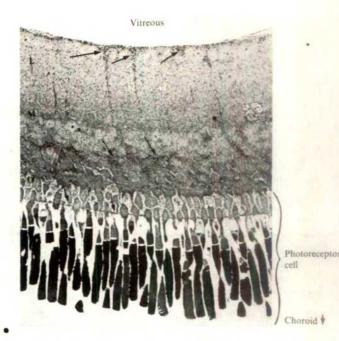


Fig. 1 Light microscope autoradiograph of frog retina showing the incorporation of <sup>3</sup>H-glutamic acid into all regions of the Müller (glial) cells. Activity can be seen over the terminal expansions of these fibres at the inner limiting membrane (L), over their cell bodies (M) and over the outer limiting membrane (O)—the proximal termination of the Müller cell (×577.50). Retinae were incubated for 30 min in the presence of 100 µCi ml<sup>-1</sup> Dt.-2-<sup>3</sup>H-glutamic acid (1.4 Ci mmol<sup>-1</sup>) at 25° C. They were then fixed in glutaraldehyde followed by osmium tetroxide and processed for autoradiography. Ilford L4 photographic emulsion was used for the autoradiography and the slides exposed for 3 d.

A representative autoradiograph obtained from a frog retina preloaded with <sup>3</sup>H glutamic acid (Fig. 1) shows there is a preferential accumulation by the Müller (glial) cells. Glutamate is also taken up by glia in both the rabbit <sup>9</sup> and rat retina (Neal and White, personal communication). In our present study, with glutamate as substrate, labelled glutamine constituted 50% of the tissue radioactivity and it would seem, therefore, that these cells are the site of a glutamate pool that produces glutamine, certainly when glutamate is used as the labelled precursor. In current terminology this would correspond to the small glutamate compartment which in brain, as well, is thought to be located in the glial cells<sup>1,2</sup>.

The formation of glutamine from glutamate within the Müller cells is compatible with the GABA-glutamine cycle proposed by Van den Berg<sup>2</sup>. This cycle might occur in the rat retina where exogenous <sup>3</sup>H-GABA is taken up by glial cells<sup>5</sup>. Such a scheme is, however, difficult to reconcile with the neuronal incorporation of <sup>3</sup>H-GABA in the frog retina<sup>12</sup>.

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## Activity of CNS depressants related to hydrophobicity

SEVERAL theories of general anaesthesia account for the depression of neuronal excitability as a depression of membrane mechanisms underlying axonal conduction1-4 or synaptic transmission<sup>5-16</sup>. The latter is more likely since general anaesthetics depress transmission in concentrations which do not block axonal conduction<sup>6,7,11,15,18</sup>. In vertebrates, excitatory postsynaptic potentials (epsps) are invariably depressed 12,15-20, while under the same conditions inhibitory postsynaptic potentials (ipsps)4.21-23 and excitatory presynaptic potentials (resulting in presynaptic inhibition) are preserved (and prolonged)<sup>24-27</sup>. Using invertebrate preparations we found<sup>28</sup> that (1) pentobarbital selectively and reversibly depresses Na+-dependent epsps without affecting either Cl<sup>-</sup>- or K<sup>+</sup>dependent ipsps, and (2) pentobarbital and other central nervous system (CNS) depressants selectively and reversibly depress postsynaptic excitation (induced by application of putative transmitters) which is coupled primarily to an increase in Na+ conductance without affecting postsynaptic inhibition (induced by application of putative transmitters) which is coupled to either Cl<sup>-</sup> or K<sup>+</sup> conductance increases. Selective depression of postsynaptic excitation would presumably lead to a decrease in the ratio of epsps to ipsps and thus account for much of the generalised decrease in neuronal activity during general anaesthesia. The observations by Meyer29 and Overton30 that general anaesthetic potency is directly proportional to the oil/water partition coefficient have prompted me to investigate the correlation between depressant activity and hydrophobicity. I report here that (1) depressant activity on invertebrate postsynaptic membranes is quantitatively correlated with hydrophobicity (r=0.982), and (2) analysis of published data from a vertebrate preparation indicates that the ratio of drug concentrations depressing axonal conduction and synaptic transmission equally is also quantitatively correlated with hydrophobicity (r = 0.96).

Dose-response curves of the inhibitory effect of six CNS depressants (chloroform, chloralose, diphenylhydantoin-DPH, ethanol, pentobarbital and urethane) on the postsynaptic voltage response and conductance change of an identified neurone in Otala lactea in response to addition of 10<sup>-5</sup> M acetylcholine (ACh) were constructed. The ACh response has an inversion potential of 0 to +10 mV and is dependent primarily on Na+ and secondarily on K+ (my unpublished observations). The dose-response curves approximately paralleled one another, suggesting that the agents each affected the ACh response through similar mechanisms. At least three and as many as ten curves were used to establish the concentration of an agent which inhibited the ACh response by 50%. The concentration producing 50% inhibition can be correlated quantitatively with the agent's hydrophobicity, as reflected by its octanol/water partition coefficient (Table 1, Fig. 1). Least

Hydrophobicity and concentration of CNS depressant Table 1 inhibiting ACh response on a molluscan neurone

Compound	log P*	Observed	log 1/C Calculated	Dev.
Ethanol	-0.32	0.70	0.85	+0.15
Urethane	-0.15†	1.23	1.04	-0.19
Chloralose	1.60	3.00	3.09	+0.09
Chloroform	2.00	3.16	3.56	+0.50
Pentobarbital	2.00	4.00	3.56	-0.44
Diphenlyhydantoin	2.50	4.16	4.15	0.01

octanol/water partition coefficient; C, molar concentration.

P, octanoi/water == \*Obtained from ref. 47. † Experimentally determined by C. Silipo and C. Hansch.

squares analysis of my data yielded equation (1) which relates the molar concentration, C, causing 50% depression of the ACh response to octanol/water partition coefficient, P

$$\log 1/C = 1.17 \log P + 1.22 \tag{1}$$

A statistical analysis, using F tests, gave:

$$\frac{n}{6} \frac{r}{0.982} \frac{s}{0.295} \frac{F_{\text{ratio}}}{F_{1,4} = 125; F_{\alpha} = 0.0005} = 106$$

where n is the number of data points, r is the correlation coefficient, s is the standard error of the estimate,  $F_{\text{ratio}}$  for significance test with  $F_{k, n-k-i}$  compared with that at a specific level of confidence,  $\alpha$ , where k is the number of independent variables and n is the number of data points used. Thus depressant activity is positively and significantly correlated with hydrophobicity over a thousandfold range of hydrophobicity and activity for the differently structured compounds, implying that steric requirements for activity are minimal.

This depression of postsynaptic excitation in invertebrates complements similar observations in the vertebrate at neuromuscular junctions<sup>1,12,31-33</sup> and on CNS neurones<sup>34-38</sup>. An increase in evoked transmitter release at neuromuscular junctions during exposure to barbiturates19,20 when epsp amplitude is reduced indicates that epsp depression here is due to a postsynaptic action. Generalised depression of postsynaptic excitation coupled primarily to Na+ (and secondarily to K+) conductance may thus account for much of the depression of neuronal excitability. Since, however, blockage of axonal conduction has been described in both invertebrates and vertebrates1-4,39, I have investigated its importance by analysing data published by Larrabee and Posternak<sup>6</sup>. They examined

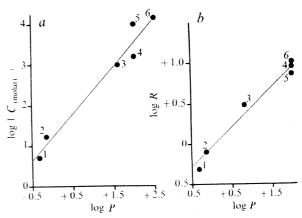


Fig 1 CNS depressant activity related to hydrophobicity. a, Loglog plot of inverse of molar concentration, C, of CNS depressants reducing ACh response on a snail neurone by 50% as a function of hydrophobicity; P, of compounds. 1, Ethanol; 2, urethane; 3, chloralose; 4, chloroform; 5, pentobarbital; 6, DPH. b. Loglog plot of ratio, R, of CNS depressant concentration depressing (by equal percentage) pre- and postsynaptically derived com-pound action potential in cat stellate ganglion as a function of hydrophobicity; P, of the agents. Data are from Larrabee and Posternak<sup>8</sup>. 1, Ethanol; 2, urethane; 3, ether; 4, chloretone; 5, chloroform; 6, pentobarbital.

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the effects of general anaesthetics on axonal conduction and synaptic transmission through the cat stellate ganglion. The concentration blocking the postsynaptic event by 50% correlated with hydrophobicity and least squares and statistical analysis of the data yield

$$\log 1/C = 1.21 \log P + 0.83 \tag{2}$$

and a correlation coefficient, r = 0.96. Equation (2) is close to that found in an analysis of data from the rabbit cervical ganglion40 by Hansch and Dunn41 who obtained

$$\log 1/C = 1.11 \log P + 0.81 \tag{3}$$

and r = 0.978. Equations (2) and (3), calculated using data from vertebrate synapses, are similar to equation (1), the major difference lying in the intercepts, which reflect the sensitivities of both the systems to the drugs and of the drug effects to the assay. The similarity inslopes of the equations indicates a similar dependency of activity on hydrophobicity.

The concentration required to block the synaptically-derived compound action potential was compared with the concentration required to block the directly recorded (presynaptic) compound action potential by an equal percentage. The ratio of the concentrations, R, was then correlated with hydrophobicity, P (data in Table 2):

$$\log R = 0.49 \log P - 0.03 \tag{4}$$

A statistical analysis gave

$$\frac{n}{6} \frac{r}{0.96} \frac{s}{0.295} \frac{F_{\text{ratio}}}{F_{1,4} = 262; F_{\alpha} = \frac{\bullet}{0000.5} = 106}$$

Equation (4) allows one to predict the ratio of depression of synaptic transmission to depression of axonal conduction from hydrophobicity. Pentobarbital, chloroform and chloretone are about ten times and ether about three times more selective for blocking synaptic transmission than for blocking axonal conduction, while ethanol and urethane appear to depress both aspects of neuronal excitation equally. Possible mechanisms include (1) a decrease in transmitter release; (2) a decreased chemosensitivity of postsynaptic receptor; (3) a blockade of the conductance mechanism coupled to the receptor, and (4) a nonspecific alteration in postsynaptic membrane properties so as to decrease the probability of excitation occurring. An increase in transmitter release at vertebrate neuromuscular junctions by these agents has been reported19,20,42 in contrast to a decrease in release at motoneurones<sup>43</sup>. (The latter may be due to an increase in presynaptic inhibition through effects on the primary afferent fibre terminals.) Nonspecific alterations in postsynaptic membrane properties do occur13,14 but at 10-100 times the concentration necessary to selectively block the receptor-coupled conductance mechanism in invertebrates and to produce effective general anaesthesia44. If the blockade of synaptic transmission in this ganglionic preparation is related to the postsynaptic effects observed in invertebrate systems<sup>28</sup> and in vertebrate neuromuscular junctions 19, 20 where alterations in end-plate currents by general anaesthetics have been described45,46, then depression of synaptic physiology in vertebrate

Table 2 Hydrophobicity and ratio of CNS depressant concentration inhibiting pre- and postsynaptic compound action potential

Compound	log P*	Observedt	log Ratio Calculated	Dev.
Ethanol	-0.32	0.30	-0.19	-0.11
Urethane	-0.15	-0.10	-0.10	0.11
Ether	+0.80	$\pm 0.48$	$\pm 0.36$	+0.12
Chloretone	2.0	$\pm 0.95$	+0.95	0.12
Chloroform	2.0	+0.85	+0.95	-0.10
Pentobarbital	2.0	+1.00	0.95	+0.05

Obtained from ref. 47.

ganglia more likely reflects a depression of the conductance mechanism coupled to postsynaptic receptors.

In summary, analysis of data derived from invertebrate and vertebrate preparations indicates that (1) agents which depress CNS excitability selectively depress epsps in invertebrates which are predominantly Na+ dependent; (2) depressant activity at a molluscan postsynaptic membrane is correlated with hydrophobicity (r = 0.982); (3) depression of a postsynaptically derived compound action potential is similarly correlated with hydrophobicity (r = 0.96, 0.978); and (4) the ratio of drug concentrations required to depress postsynaptic excitation and presynaptic axonal conduction equally is also positively correlated with hydrophobicity (r = 0.96). The results suggest that much of the depression of CNS excitability observed with those agents commonly used as general anaesthetics is due primarily to a selective depression of postsynaptic excitation through a postsynaptic mechanism and secondarily to a depression of axonal conduction by the more hydrophilic agents (ethanol and urethane). In addition, the mechanisms underlying the effects of general anaesthetics on presynaptic physiology in the vertebrate and the relative importance of presynaptic inhibition in the depression of CNS excitability by these agents requires further investigation. Finally, the roles played by hydrophobicity in determining whether the attack of these agents is directed either to voltage-independent (postsynaptic receptor-coupled) and/or to voltage-dependent (axonal) monovalent conductance mechanisms (and to presynaptic physiology) remains to be elucidated.

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P, octanol/water partition coefficient; C, molar concentration.

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## Temperature-dependent interconversion of histamine H<sub>1</sub> and H<sub>2</sub> receptors in guinea pig ileum

HISTAMINE receptors mediating changes in gastric secretion are resistant to blockade by most antihistamines1, and this led Ash and Schild' to propose two classes of receptors, H typified by the system in guinea pig ileum or bronchi, and H<sub>2</sub> responsible for effects on atria and gastric secretion. This concept is supported by the demonstration that, in contrast to most antihistamines, a series of substituted imidazole compounds are competitive antagonists of the H2 receptor with negligible action on H1 (refs 3, 4).

During studies of the effects of changes in temperature on the blocking action of some competitive H1 antagonists, we found that below 18° C promethazine ceased to be competitive and appeared to act non-competitively. Since promethazine can act as a non-competitive antagonist of the H<sub>2</sub> receptor in atria<sup>5</sup> this raised the possibility of some transformation of the H1 to the H2 receptor at lower temperatures. We report here studies which support this view.

Isolated strips of the longitudinal smooth muscle of guinea pig ileum6 were set up in organ baths containing

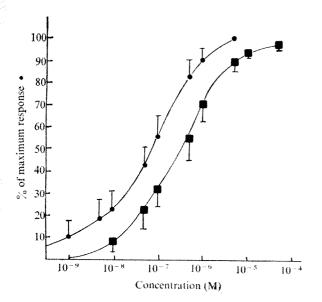


Fig. 1 Dose-response curves for histamine at 12° C. Bars represent standard error from five experiments.  $\bullet$ , Control;  $\bullet$ , after treatment with  $7.5 \times 10^{-3}$  M metiamide.

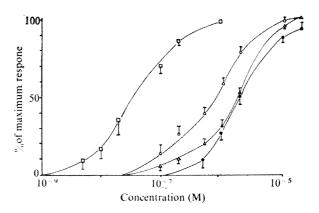


Fig. 2 Dose-response curves for histamine. Bars represent standard errors from six experiments. □, Control response to histamine at 37° C; △, after  $5 \times 10^{-8}$  M tripelennamine at 37° C; O, after  $5 \times 10^{-8}$  M tripelennamine at 12° C; •, after  $5 \times 10^{-8}$  M tripelennamine and  $7.5 \times 10^{-5}$  M metiamide at 12° C.

Tyrode solution gassed with 95% O2:5% CO2 and allowed to equilibrate for 1 h at 37° C. Contractions were recorded using either a frontal writing lever and a kymograph, or a linear motion transducer (Hewlett Packard 7DCDT) and a polygraph (Grass 5P1). Responses to histamine in this preparation were stable for several hours both at 37° C and 12°C, although some changes in shape of the doseresponse curve were observed at lower temperatures. At 37° C the H<sub>2</sub> antagonist metiamide, at concentrations up to  $2 \times 10^{-4}$  M, had no effect on the responses to histamine, in agreement with the findings of Black et al.4. At 12° C  $7.5 \times 10^{-3}$  M metiamide produced a parallel shift in doseresponse curve to the right (Fig. 1). Rewarming the tissue to 37° C resulted in some persistence of blockade, but after 1 h responses were similar to those obtained initially in the absence of metiamide.

To demonstrate that the effects were specific for the histamine receptor these experiments were repeated using acetylcholine as the agonist. Metiamide produced no antagonish of the responses to this agent at either 37° C or 12° C at concentrations up to 10<sup>-4</sup> M.

The H<sub>1</sub> receptor antagonists usually have some action on H<sub>2</sub> receptors, but tripelennamine is reported to have very high selectivity for  $H_1$  (ref. 7). Tissues treated with  $5 \times 10^{-8}$ M tripelennamine at 37° C showed the expected shift in doseresponse curve to the right. At 12° C, and in the continued presence of tripelennamine, some reversal of blockade occurred and this could be blocked again with metiamide (Fig. 2). As this recovery was unaffected by 10<sup>-6</sup> M atropine it is unlikely to have arisen from any action of histamine on release of acetylcholine. No sensitisation to acetylcholine was observed at lower temperatures, after treatment with either atropine or tripelennamine.

The ability of metiamide to antagonise the histamineinduced responses of this preparation at lower temperatures and the reduced ability of tripelennamine to block contractions under these circumstances suggests that at 12° C some receptors were transformed from H<sub>1</sub> to H<sub>2</sub>. This is in many ways similar to the findings of Kunos et al.\* concerning the interconversion of the  $\alpha$  and  $\beta$  receptors in heart, but we are unable, to present, as they did, evidence based on selective actions of agonists since selective H<sub>1</sub> and H<sub>2</sub> agonists were not available to us. Betazole is reported to be about ten times as active on sites regarded as H<sub>2</sub> receptors, and it would thus be expected that the tissue would show increased sensitivity to betazole at lower temperatures. A small increase in sensitivity to betazole was observed in three of six experiments and this was antagonised with metiamide, but control experiments indicated that betazole shows marked tachyphylaxis particularly at

low temperatures, and this observation makes study of the H<sub>1</sub> to H<sub>2</sub> interconversion using this agonist difficult to evaluate.

In conclusion, our data suggest that at low temperatures some H1 receptors of guinea pig ileum convert to a form similar to the H<sub>2</sub> receptor. The concept of a change in the properties of receptors as a result of changes in temperature is not unexpected in view of the concept of membrane phase transitions supported by enzyme studies10. Furthermore, Rocha e Silvaii has suggested that the properties of the histamine receptor at temperatures above 20° C are different from those at lower temperatures.

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### Astrocyte-specific protein and radial glia in the cerebral cortex of newborn rat

Here we show that radial glia directing neuronal migration in the cerebral cortex during development can respond to injury with the production of a protein specific to astrocytes, and may thus be regarded as an early form of the mature astrocyte.

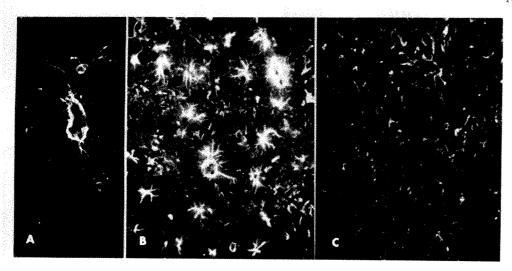
The migration of nerve cells from their site of origin in germinal zones to their final destination is a general rule in brain development, the granular layer of the hippocampus being the only possible exception. Migrating neuroblasts in the cerebellar and cerebral hemispheres are in close contact with radially oriented glial processes1-3. Such close relationship suggests that glia play a role in directing radial migration of neurones through complex regions of the brain which contain bundles of nerve fibres in various orientations early in development. In the cerebellum, Bergmann glia provide the guidelines for neurones migrating inwards from the external granular layer. They persist in adult life as a distinct form of fibrous astrocytes with straight parallel processes crossing the molecular layer at right angles and reaching the surface of the cerebellum4. During development of the cerebral hemispheres the radially oriented glial cells directing the outward migration of neurones from the ventricular zone have been observed in many species by the Golgi method and more recently by electron microscopy in the monkey and the rat2,3. These cells are not observed in mature brain and have been interpreted as spongioblasts (pluripotential glial precursors3), or as transient forms of astroglia transforming into astrocytes of more typical appearance later in development<sup>2.5</sup>.

The glial fibrillary acidic (GFA) protein is brain specific but not species specific and is selectively located in fibrous astrocytes<sup>6,7</sup>. The appearance and distribution of neuroglia using immunofluorescence with GFA protein antibodies correspond to those described by Weigert4 with his method for fibrous astrocytes. By immunofluorescence, Bergmann gliai processes transversing the external granular layer and molecular layer of the cerebellum are first demonstrated on the third postnatal day in the rat and mouse, thus before the beginning of neuronal migration in these species<sup>8.9</sup>. In a similar study of astroglial differentiation in the rat cerebral hemipheres', radial glial processes were only demonstrated in the granular layer of the hippocampus in the first weeks of postnatal development. Immunofluorescent fibres were first observed on the 18th embryonic day in the medial wall of the lateral ventricles and they had no radial orientation.

After injury the astrocytes become larger with increasing gliofibrillary content. In a study of the glial reaction during development, the brains of adult and newborn rats were stabbed unilaterally in the lateral frontal region. This was done with a scalpel through the intact skull in the parasaggital plane. Animals were killed at intervals ranging from 2 d up to 2 months. Air-dried coronal cryostat sections were used for immunofluorescence studies. Adjacent sections were stained with toluidine blue. GFA protein antisera previously obtained from New Zealand albino rabbits7 were used. The rabbits had been injected with human GFA protein purified from multiple sclerosis plaques, a severely gliosed tissue markedly enriched in GFA protein<sup>10,11</sup>. The immunofluorescence test was carried out according to Coons indirect procedure, as previously described7. Control sections were incubated with preimmunisation sera and immune sera absorbed with GFA protein. GFA protein antisera and preimmunisation sera were used in 1/50 dilutions. At this titre essentially no fluorescein staining was seen in control sections. The fluorescein-labelled goat anti-rabbit globulin serum was used in 1/10 dilutions.

In toluidine blue stained sections the damage to the lateral frontal cortex was extremely limited and confined to the vicinity of the blade track. In the subjacent white matter the damage was more extensive and in newborn animals resulted in a necrotic and cavitating lesion bordering the frontal cortex on the medial surface of the hemisphere.

The astrocytic reaction to injury was studied by immunofluorescence with GFA protein antiserum in the frontal cortex. In the rat isocortex most astrocytes are protoplasmic and do not contain GFA protein in detectable amounts. In the adult animal immunofluorescence is essentially confined to the external glial membrane on the surface of the brain and to the glial membranes surrounding small arteries and veins (Fig. 1A). None of these structures is immunofluorescent in the newborn rat. A continuous external glial membrane is first observed by immunofluorescence on the 9th day, while the perivascular glial membranes form later (9-18 d) (ref. 7). In the adult rat 2 d after stabbing protoplasmic astrocytes had switched to GFA protein production and thus became brightly immunofluorescent with GFA protein antiserum (Fig. 1B). This change, still present 2 months after injury, was not confined to the vicinity of the wound but extended through wide areas of the cortex in the stabbed hemisphere. In newborn rats sacrificed 2 d after stabbing, at a time when no immunofluorescence is normally present, immunofluorescent fibres had appeared around the blade track in the lateral frontal cortex (Fig. 1C) and in the medial frontal cortex overlying the necrotic lesion in the white matter (Fig. 2). While in the lateral cortex these processes were irregularly oriented. in the medial cortex they had the appearance of thin parallel processes transversing the cortex at right angles to the surface. These radial processes had disappeared 13 d after injury. At that time immunofluorescent fibres had the same irregular orientation in the medial and lateral cortex. In 1-month-old



Astrocytic response to injury in the middle layers of the rat frontal cortex 2 d after stabbing. In the normal adult rat (A) immunofluorescence with GFA protein antiserum is essentially confined to the glial membranes surrounding small arteries and veins. After stabbing (B) the protoplasmic astrocytes which normally do not stain become brightly immunofluorescent. This is an extensive reaction involving large areas of the stabbed hemisphere. In the newborn rat (C) the reaction is already present but less intense (no immunofluorescence is normally seen (×14.5) at this stage of development). Immunofluorescence with GFA protein antiserum.

animals which had been stabbed at birth, the reactive astrocytes in the cerebral cortex were identical to those observed in mature animals following the same type of lesion, that is they appeared as conventional astrocytes in Cajal preparations. The astrocytic. reaction had become very extensive and was also present in the cortex of the unstabbed hemisphere.

Radial glial cells in the medial frontal cortex of the newborn rat behave like protoplasmic astrocytes in mature brain. that is, they are switched to GFA protein production as a response to injury. Radial glia may thus be considered as an · early form of astrocytes that transforms into astrocytes of more conventional morphology in the first weeks of postnatal development. It may be noted that radial glia persist through adult life as the main type of supporting glia in lower vertebrates12. As is the case with astrocytes in mammalian brain, radial glial cells in lower vertebrates may not contain GFA protein in detectable amounts. Thus, radial glia could be demonstrated by immunofluorescence in the optic tectum of the turtle, but not in the optic tectum of the goldfish and frog6.

It seems that astrocytic determination, as opposed to GFA protein expression, is an early phenomenon in development. According to Rakic (personal communication), some of the elongated columnar neuroepithelial cells spanning the entire thickness of the telencephalic wall, develop vascular attachments as early as 48 d after conception in the monkey (gestation time 165 d). The constraints imposed by the mesenchymal interaction are probably a major factor restricting the pluripotential neuroepithelium to the role of supporting glia (radial astrocytes). GFA protein expression occurs much later in the



Fig. 2 Radial glia in the newborn rat (deep layers of the medial frontal cortex) 2 d after stabbing. Radial glia is not stained by immunofluorescence with GFA protein antiserum in normal development. W, injured white matter underlying the medial frontal cortex. Immunofluorescence with GFA protein antiserum

course of normal development, most likely as a result of permissive tissue interactions.

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## Apomorphine and morphine stimulate prostaglandin biosynthesis

Among the acute pharmacological effects of apomorphine and morphine are emesis, hyperthermia and hyperglycaemia1,2, Because administration of prostaglandins also elicits each of these effects3-10, we have investigated whether apomorphine or morphine stimulates prostaglandin biosynthesis. Here we describe experiments showing that both drugs stimulate, although possibly in a somewhat different way, the prostaglandin (PG) synthetase of bull seminal vesicles.

Bull seminal vesicles, kept for not more than four weeks at -20° C, were homogenised for 1-2 min at 4° C in three volumes of 50 mM phosphate buffer (pH 7.4) containing 1 mM EDTA. The strained homogenate was centrifuged at 600g for 15 min and the supernatant separated and stored under nitrogen at  $-20^{\circ}$  C. Two different preparations of this supernatant were used in the course of these experiments, with comparable results. The incubation mixture consisted of 500 µl of the supernatant in 2 ml of 50 mM phosphate buffer, with or without apomorphine hydrochloride or morphine sulphate. The reaction was started by addition of sodium arachidonate (final concentration 61 µM) and the tubes were

aerated with gentle shaking. After 15 min, the reaction was stopped by adding 2 ml 0.2 M citric acid and the mixture was extracted with 16 ml ethylacetate. After centrifugation at 600g for 5 min, 11 ml of the ethylacetate layer was evaporated to dryness in vacuo, the residue dissolved in 500 µl ethanol, and 100 µl aliquots were diluted 50-fold in Krebs' solution. Volumes of not more than 0.25 ml of these aqueous ethanol extracts were assayed against reference PGE2 on rat isolated stomach fundus strip, superfused at 5 ml min-1 with Krebs' solution, containing mixed antagonists of acetylcholine, catecholamines, histamine and 5-hydroxytryptamine<sup>11</sup>, and aerated with oxygen containing 5% carbon dioxide.

In some experiments, aliquots (125 µl) of the ethanol solution were chromatographed in the A I system<sup>12</sup> on silica gel plates with PGE2 and PGF20 markers. The areas corresponding to each prostaglandin were separately extracted with ethanol and the residues after evaporation were redissolved in 200  $\mu$ l ethanol and diluted 25-fold with water for bioassay. Assays were then performed against reference PGE, and PGF, on rat isolated fundus strip and colon, superfused in series. The concentrations of drugs are expressed as those of active acid

In control experiments, we found that addition of apomorphine (100 or 400  $\mu g$  ml  $^{-1})$  or morphine (10 or 100  $\mu g$  ml  $^{-1})$   $_{\bullet}$ to the boiled enzyme before incubation did not affect the blank assay values for total prostaglandin, indicating that the assay was not affected by drug being carried through the extraction process. In other control experiments, 3H-PGE2 and 3H-PGF2a were incubated with unboiled or boiled enzyme for 15 min, extracted, separated by thin layer chromatography, and assayed. In this procedure, <8% of the PGE<sub>1</sub> and no PGF<sub>2</sub> $\alpha$ were lost during incubation with unboiled enzyme preparation. We concluded that a very large part of the increases in net production of prostaglandins found in the presence of apomorphine or morphine could be attributed to increased prostaglandin biosynthesis and not to protection of prostaglandin from enzymatic breakdown.

PG synthetase was incubated with arachidonic acid in the absence of drug, or in the presence of different concentrations of apomorphine, and the yield of prostaglandin-like material assayed against PGE. After subtracting the amount of PGlike material found in control tubes containing boiled enzyme, the assay value was taken to represent PG produced by the action of synthetase during incubation. With unboiled enzyme in the absence of drug, a mean of 1,137 ng (s.e.  $\pm$ 129) of prostaglandin was produced. When a pomorphine (0.1–100  $\mu g$ ml-1) was present, mean PG production exceeded the control value by 1.26-fold to 4.42-fold (Fig. 1). In the presence of apomorphine (1-100 µg ml<sup>-1</sup>), mean PG production sharply increased with increasing concentration of drug (slope, 1.64; s.e.,  $\pm 0.379$ , P < 0.001). A higher concentration of apomorphine (400 µg ml-1) inhibited PG production. From the mean concentration/response line it was calculated that 1.71 µg ml<sup>-1</sup> (limits, 1.33-5,44) of apomorphine were required to increase the PG production by a factor of 1.5 (SC 50).

In similar experiments, morphine (0.01-100 µg ml<sup>-1</sup>) stimulated PG production by 1.21-fold to 2.17-fold (Fig. 2). Compared with apomorphine, morphine had a concentration/ response line of low slope; but this was statistically significant (slope, 0.149; s.e.,  $\pm 0.045$ ; P < 0.005). The SC<sub>50</sub> value for morphine was 1.59  $\mu$ g ml<sup>-1</sup> (limits, 0.0855-52.4).

In both experiments with each drug, in which prostaglandins produced were separated by thin layer chromatography before assay, apomorphine and morphine stimulated production of both PGE<sub>2</sub> and PGF<sub>2a</sub>. For example, in one experiment, apomorphine, at 100 μg ml<sup>-1</sup>, increased production of PGE<sub>s</sub> by 2.47-fold and that of PGF<sub>2a</sub> by 1.66-fold; and morphine, at 1 μg mI<sup>-1</sup>, increased production of PGE<sub>2</sub> by 1.30-fold and of  $PGF_{2\alpha}$  by 1.27-fold.

In three independent experiments, chlorpromazine hydrochloride and sodium acetylsalicylate were tested for their ability to inhibit the stimulation of PG synthetase induced by

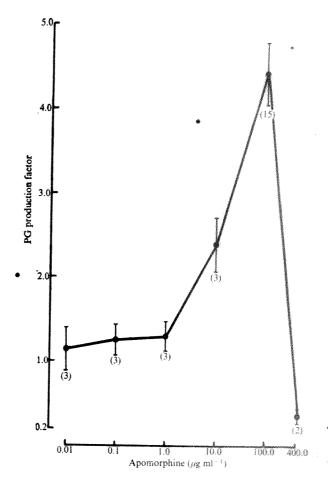


Fig. 1 Stimulation by apomorphine of the PG synthetase of built seminal vesicles. The production factor is the factor by which prostaglandin production in the presence of apomorphine exceeded that in its absence in reference tubes in the same experiment. The results of 2-15 independent experiments are expressed as the mean production factor  $\pm$  s.e. In brackets, the number of values used to calculate each mean

100 µg ml<sup>-1</sup> of apomorphine. Chlorpromazine, at 0.9, 9 and 90 μg ml<sup>-1</sup>, reduced apomorphine stimulation in a dose-related way, by mean percentages of 3.1, 25.8 and 60.5 respectively. Acetylsalicylate reduced PG production both in the presence and absence of apomorphine.

These experiments show that apomorphine and morphine stimulate the prostaglandin synthetase of bull seminal vesicles in the absence of the cofactors, glutathione and hydroquinone, that are usually added. Experiments now in progress show that such stimulation can also occur in the presence of these cofactors<sup>13</sup>. Other experiments in progress show that the stimulation by apomorphine and morphine of PG synthetase is not restricted to the enzyme from bull seminal vesicles, but occurs also with synthetase from rabbit brain14.

The low slope of the concentration/response relationship for morphine could arise from various causes; for example, the action of morphine could be limited by a factor independent of the drug concentration; or, again, morphine might activate processes with opposed concentration/response relationships. The difference in this respect from apomorphine may indicate a difference in the mechanism of action of morphine and apomorphine on this preparation.

Morphine is known to act centrally in producing emesis 16,18, hyperthermia<sup>17</sup> and hyperglycaemia<sup>18,19</sup>. Apomorphine, too, exerts these effects by acting on the brain (ref. 15 and W. Feldberg, unpublished). In all the experiments reported above, apomorphine and morphine stimulated prostaglandin biosynthesis at concentrations less than those attainable in the brain with tolerated doses of either drug<sup>20,21</sup>. Prostaglandins are produced in the brain<sup>22</sup>, and E-type prostaglandins are known to act there to elicit fever 7.8. It is not known, however,

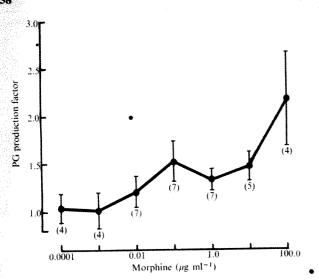


Fig. 2 Stimulation by morphine of the PG synthetase of bull seminal vesicles. Values are the means of 4-7 independent experiments. Other details as in Fig. 1.

whether prostaglandins can elicit vomiting or hyperglycaemia also by a central action. We therefore propose that the hyperthermic effects of apomorphine and morphine, and possibly also their emetic, hyperglycaemic and allied effects, may be due to the stimulation of prostaglandin biosynthesis. This proposition requires that chlorpromazine, which inhibits apomorphine-induced vomiting in the dog23,24, should inhibit the stimulation by apomorphine of PG synthetase; and we have found this to be so.

It has recently been proposed that morphine may exert its analgesic or allied effects by inhibiting the stimulation by E prostaglandin of cyclic AMP formation<sup>25</sup>. Thus, diverse interactions with prostaglandin mechanisms might explain some depressant and excitatory effects of morphine on the nervous system and provide the basis for a mechanism of tolerance/ dependence.

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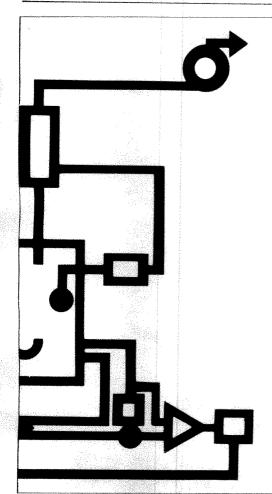
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## Inhibition of glycoprotein and glycolipid synthesis in hamster embryo cells by cytosine arabinoside and hydroxyurea

THE nucleoside, 1-β-D-arabinofuranosylcytosine (ara-C) and the unrelated compound, hydroxyurea, are known to inhibit DNA replication in bacterial and animal cells1-3. Arabinofuranosyl cytidine-5'-triphosphate (ara-CTP) specifically interferes with the action of DNA polymerase II in bacterial cell extracts4, whereas hydroxyurea affects the ribohucleoside diphosphate reductase reaction5. We have shown that ara-C and hydroxyurea bring about transformation of hamster embryo cells in tissue culture. Further, it has also been demonstrated that ara-C inhibits the incorporation of 3H-D-glucosamine (a precursor of N-acetylneuraminic acid) into glycoproteins and glycolipids of hamster embryo fibroblasts and transformed cells7. In continuing these experiments we have been interested in, first, determining the site or sites of action of ara-C (presumably as its triphosphate) in the cell and second, studying other inhibitors of DNA replication as to their possible effect on glycoprotein and glycolipid synthesis.

To compare the effect of ara-C and other chemicals on both DNA replication and glycoprotein/glycolipid synthesis, either secondary cultures of hamster embryo fibroblasts or transformed hamster embryo cells were used as the test system6. Cells were exposed to the different chemicals for 24 h, and the incorporation of 14C-thymidine and <sup>3</sup>H-p-glucosamine measured into acid-precipitable material at selected times during this period.

As expected, ara-C and hydroxyurea are potent inhibitors of DNA replication, while FUdR and the carcinogenic hydrocarbons were less effective in this respect (Table 1). With regard to the incorporation of 3H-D-glucosamine (glycoproteins and glycolipids), ara-C inhibited by 86% in normal cells and 50% in transformed cells (Table 1). Higher inhibitions have been observed with transformed cells in further experiments. Hydroxyurea brought about a 70% inhibition of incorporation in normal cells and a  $75\,\%$ inhibition in transformed cells, FUdR and the polycyclic hydrocarbons inhibited incorporation but to a much lesser extent (20-30%). Figure 1 shows the time curve of incorporation of 14C-thymidine (Fig. 1a) and 3H-D-glucosamine (Fig. 1b) into DNA and glycoproteins/glycolipids of trans-



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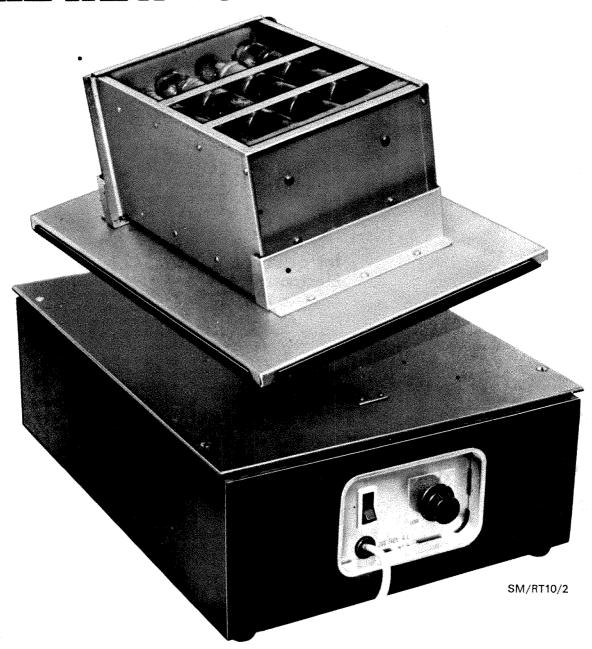
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Table 1 The effect of ara-C, hydroxyurea and other chemicals on the incorporation of <sup>14</sup>C-thymidine into DNA and <sup>3</sup>H-D-glucosamine into glycoproteins/glycolipids of cells in tissue culture

	Incorporation <sup>14</sup> C-thymidine		% Inhibition		Incorporation <sup>3</sup> H	% Inhibition		
Custom	(c.p.m./mg protein $\times$ 10 <sup>-3</sup> )		m e 1 37 1		(c.p.m./mg protein × 10 <sup>-3</sup> )		nn 0 1	***
System	Transformed	Normal	Transformed	Normal	Transformed	Normal	Transformed	Normal
Control	388	567			200	122	(g <sub>i</sub> )mle(g)map.	opionina
Ara-C (10 <sup>-3</sup> M)	11.4	89.7	97%	84%	100	17.2	50%	86%
Hydroxyurea (10 <sup>-2</sup> M)	4.9	8.2	99 % 39 %	99%	49	25.4	75%	86 % 79 % 27 %
FUdR (10 <sup>-3</sup> M)	235	377	39 %	43%	138	89	31 %	27%
Methylcholanthrene (10 µg ml <sup>-1</sup> )	277	495	28 %	13%	157	107	22%	21 %
Benzpyrene (10 µg ml <sup>-1</sup> )	267	461	31 %	43 % 13 % 19 %	138	106.5	31 %	21 % 23 %

Experimental details as for Fig. 1. Incubations were at 37° C for 24 h. Four other individual experiments gave similar results.

formed cells and the effect of ara-C and hydroxyurea on these reactions. The results indicate continuous inhibition of incorporation of both radioactive precursors over 24 h.

The observations recorded in Table 1 and Fig. 1 on the simultaneous inhibition of DNA replication and glycoprotein/glycolipid synthesis by ara-C and hydroxyurea in particular, suggest some correlation between these two synthetic pathways. It is worth mentioning that ara-C (10<sup>-3</sup> M) inhibits <sup>3</sup>H-uridine incorporation by 3% (normal cells) and 11% (transformed cells) after 24 h of incubation. Protein synthesis was stimulated 3% (normal cells) and inhibited by 7% (transformed cells) at the same time period. On the other hand, hydroxyurea (10<sup>-2</sup> M) inhibited <sup>3</sup>H-uridine incorporation by 30% (normal cells) and 17% (transformed cells) after 24 h of incubation. Protein synthesis was inhibited by 12% (normal cells) and 3% (transformed cells) respectively at the same time period. Ara-C

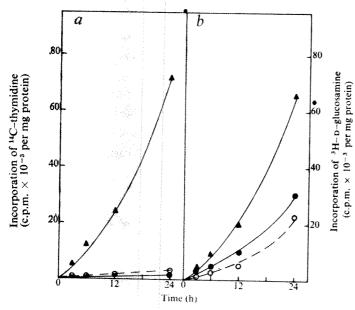


Fig. 1 Effect of ara-C and hydroxyurea on the incorporation of <sup>14</sup>C-thymidine into DNA and <sup>3</sup>H-D-glucosamine into glycoproteins and glycolipids of transformed hamster embryo cells. Incubations were carried out in Falcon Petri dishes (5.5 cm diameter) containing 5.0 × 10<sup>5</sup> cells in 3 ml of growth medium (Eagle's Minimal Essential medium containing Earles salts, 10% calf serum, penicillin and streptomycin) at 37°C in a 5% CO<sub>2</sub> atmosphere. Incorporation experiments were commenced after approximately 24 h, by which time the cells had half mono layered. Ara-C was present at a concentration of 10-<sup>3</sup> M and hydroxyurea at 10-<sup>2</sup> M. Each Petri also contained 1.0 μC <sup>14</sup>C-thymidine and 0.5 μC <sup>3</sup>H-D-glucosamine per ml incubation medium. At selected times, the medium was removed, the cells rinsed with PBS, and the cell pellet homogenised in 1 ml of water by means of a syringe using a No. 21 needle. Aliquots were taken for counting and protein determination<sup>13</sup>. For counting, samples were precipitated with cold 10% trichloroacetic acid (TCA), transferred to Whatman GF/C filters and washed with 25 ml of cold 5% TCA. Counting was carried out in a Scintillation Spectrometer. a, <sup>14</sup>C-thymidine incorporation. Δ, control; ○, ara-C; ♠, hydroxyures.

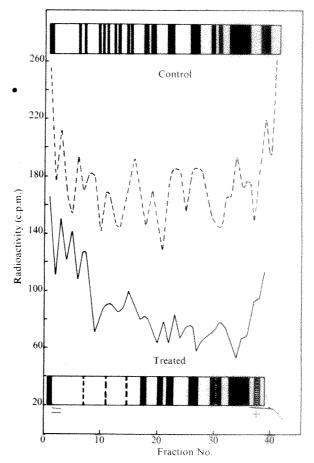


Fig. 2 Polyacrylamide gel electrophoresis of cell membrane glycoproteins. Cells were grown in Roux Flasks as described for Fig. 1. <sup>3</sup>H–D–glucosamine was present at 1.0 μCi per mi incubation medium. Ara–C when present was at a concentration of 10<sup>-3</sup> M. After 24 h at 37° C, cells were washed with PBS and removed from the glass by treatment with PBS containing 0.02% (w/v) EDTA for 30 min at 37° C. Cell membranes were isolated by a modified method based upon the procedures of Neville<sup>14</sup> and Graham<sup>15</sup>. Glucose–6–phosphatase and 5'–nucleo-tide activities were assayed according to the methods of Swanson<sup>16</sup> and Heppel and Hilmore<sup>17</sup>, respectively. Polyacrylamide gel electrophoresis was carried out in buffers according to Jones<sup>18</sup>. Protein was stained with Amido Black and gel silces were treated with H<sub>2</sub>O<sub>2</sub> before counting in Bray's solution.—Radioactivity in glycoproteins of control cells; ——, radioactivity in glycoproteins of cells treated with 10<sup>-3</sup> M ara-C.

has also been found to have no effect on the incorporation of CTP into the 3'-terminus of tRNA catalysed by the nucleotide-incorporating enzyme. The latter experiments were carried out *in vitro* with rat liver enzymes and chemically synthesised ara-CTP (T.S-B., and L. Parkin, unpublished). Further, as previously reported<sup>7</sup>, ara-C does not affect the incorporation of <sup>14</sup>C-choline into phospholipids of normal or transformed hamster embryo fibroblasts.

We compared the patterns of labelling of glycoproteins of the cell membranes of both normal and ara-C treated cells

Table 2 The effect of ara-C and hydroxyurea on the transfer of sugars from their respective nucleotide carriers to glycoproteins of the cell membrane

Ir	acorporation into TCA	precipitable material		
	(c.p.m./mg	protein)		
System	Control	Plus 10-3 M ara-C	% Inhibition	
<sup>14</sup> C-UDP-galactose	1,509	730	52	
<sup>3</sup> H-UDP-glucose	1,205	1,220	**************************************	
<sup>14</sup> C-UDP-N-Ac-glucosamine	775	216	73	
<sup>14</sup> C-CMP-sialic acid	523	160	69	
		Plus 10 <sup>-2</sup> M hydroxyurea		
<sup>14</sup> C-UDP-galactose	1,250	620	50	
<sup>3</sup> H-UDP-glucose	4,950	5,050	-Laterature	
<sup>14</sup> C-UDP-N-Ac-glucosamine	580	240	59	
<sup>14</sup> C-CMP-sialic acid	740	220	70	

Transformed cells were pre-incubated in Petri dishes until approximately half monolayered as described for Fig. 1. After removal of the medium, cells were rinsed with 0.01 M Tris-HCl (pH 7.6) + 0.15 M NaCl and then incubated in a buffer containing sucrose (0.15 M); Tris-HCl, pH 7.6 (40 mM); KCl (15 mM); MgCl<sub>2</sub> (6.6 mM) and MnCl<sub>2</sub> (6.6 mM). Radioactive nucleotide sugars were present at 1 µCi per 3.0 ml incubation medium. Incubation was for 1 h at 37° C. Cells were processed for radioactivity and protein content as described for Fig. 1.

For this purpose, cells with ara-C (10<sup>-3</sup> M) and without ara-C were incubated for 24 h with 3H-D-glucosamine. Thereafter, cell membranes were isolated and washed prior to analysis by means of polyacrylamide gel electrophoresis. The results of these experiments are shown in Fig. 2. Definite differences are seen as regards the distribution of proteins between the normal and ara-C-treated cells. Also, the labelling of the glycoproteins is generally much lower in the ara-C-treated cells as compared with the control cells. Similar results have been obtained with the glycoproteins from cells treated with hydroxyurea.

Various research workers have shown that labelled sugars can be transferred from their activated nucleotide carriers to receptors on the cell surface<sup>8-18</sup>. It is assumed that cell surface glycosyl transferases carry out these reactions. It was of considerable interest to ascertain whether ara-C and hydroxyurea were capable of interfering with these transferase reactions at the cell surface or not. The transfer of labelled D-galactose, D-glucose, N-acetyl Dglucosamine and sialic acid from their respective nucleotide carriers to cell surface glycoproteins was carried out as described in Table 2. It can be seen that ara-C and hydroxyurea inhibit the transfer of galactose, N-acetyl D-glucosamine and sialic acid to a considerable extent, whereas the transfer of glucose does not seem to be affected.

The inhibitions observed could possibly be due to direct interaction of the inhibitors with the glycosyl transferase enzymes of the cell surface (see Table 2). Preliminary experiments with isolated microsomes from transformed hamster embryo cells and rat liver have shown that ara-C (10<sup>-3</sup> M) has no effect on the transfer of labelled sugars from their respective nucleotide carriers to endogenous receptors present in the microsomes. On the other hand, ara-CTP (10-3 M) strongly inhibits the transfer of sugars from their carriers to microsomes. The transfer of UDP-galactose was inhibited by approximately 80%, UDP-N-acetyl-glucosamine and GDP-mannose both by 60% and CMP-sialic acid by 25%. Hydroxyurea (10<sup>-2</sup> M) also inhibited direct transfer of the sugars but to a lesser extent. These preliminary results suggest a direct interference with the glycosyl transferases by the inhibitors discussed. They also suggest that the inhibition of glycoprotein/glycolipid synthesis observed is not due to indirect effects operating through the inhibition of DNA replication.

In the case of ara-C, where presumably the effective inhibitor is ara-CTP, inhibition occurs through direct interaction with the enzyme activating N-acetylneuraminic acid (NANA). Work in our laboratory (L. Parkin and A.O.H., unpublished) with chemically synthesised ara-CTP has shown that the nucleotide inhibits activation in rat liver nuclear extracts11 and with the partially purified enzyme from hog submaxillary glands12. Maximum inhibition has been of the order of 50-60%. We have also shown pre-

viously that ara-CMP (10<sup>-3</sup>-10<sup>-5</sup> M) inhibits the transfer of NANA from CMP-NANA to receptors by approximately 20%. It seems therefore that ara-C as its triphosphate is inhibiting both the activation and subsequent transfer of NANA to receptors. The effect of ara-CTP on the transfer of other activated sugars to receptors in isolated microsomes is of considerable interest. These are direct effects and are under study at the moment.

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## Assignment of the human gene for galactokinase to chromosome 17

EVIDENCE presented here and in the report by Elsevier et al.1 mutually establishes the assignment of the human gene for galactokinase (EC 2.7.1.6), the enzyme which converts galactose to galactose-1-phosphate, to chromosome 17. This assignment is based on a syntenic relationship between the genes for thymidine kinase and galactokinase.

Hybridisation of rodent and human somatic cells and the preferential loss of human chromosomes which segregate in the resultant hybrid clones has become an important procedure for assigning human genes to specific chromosomes2. When the HAT selection system3 is used to select hybrids produced by fusion of a thymidine kinase-deficient rodent cell line and a thymidine kinase-positive human cell line, the human gene for thymidine kinase is retained since activity of this enzyme is required for cell survival in HAT medium4. Using this selection system the gene for human thymidine kinase was first assigned to a group E chromosome<sup>5,6</sup>; later specifically to chromosome 17 (ref. 7), and more recently to the long arm of this chromosome8. When HAT selected hybrids are placed in medium containing 5-bromodeoxyuridine (BrdU), the incorporation of this thymidine analogue into DNA, made possible by thymidine kinase activity, is lethal, thereby producing selection against cells retaining the thymidine kinase gene9. Furthermore, the hybrid cells which can grow in medium containing BrdU, have lost the human chromosome 17 (ref. 9).

The hybrid clones analysed for human galactokinase activity were derived independently from five separate fusion experiments involving mouse (CI1D) and human (KOP-2, LNSV and W18Va2) parental cell lines. These parental cell lines and the procedures used for cell fusion have been described before. After fusion, hybrid cells were maintained in HAT selective medium and subsequently cloned. Each clone was derived from a different hybrid cell colony. Nine of the HAT selected hybrid cell clones were transferred to medium containing 30 µg ml<sup>-1</sup> BrdU and maintained in it for at least 30 passages. Mouse and human chromosomes were identified in each of the hybrid cell clones by Giemsa-banding as previously described. A minimum of 20 metaphases from each hybrid cell clone was analysed to identify the human chromosomes present.

Thirty-two human-mouse hybrid cell clones were tested for the presence of human galactokinase. Extracts of these hybrid clones were electrophoresed in starch by the methods of Tedesco et al.<sup>10</sup>. In this procedure, enzyme activity is localised by staining the gel with a reaction mixture<sup>10,11</sup> containing 0.5% Ionagar No. 2 (Consolidated Laboratories Inc.) 0.2 M Tris-HCl, pH 8.0, 7 mM β-mercaptoethanol, 2 mM MgCl<sub>2</sub>, 0.94 mM NADP, 0.6 mM UDP-glucose, 0.21 mM glucose-1, 6-diphosphate, 0.4 mM galactose, 6.6 mM ATP, 0.1 U ml<sup>-1</sup> yeast galactose-1-P uridyl-transferase (Sigma), 0.2 mg phosphoglucom<sup>1</sup>1tase (Boehringer-Mannheim), 0.2 mg glucose-6-phosphate-dehydrogenase (Boehringer-Mannheim), and 0.2 mg

Table 1 Syntenic relationship between human galactokinase and thymidine kinase

	The William Control of the Control o	
	Human Gk+	Human Gk-
HAT selected clones	23	0
BrdU selected clones	U	

6-phosphogluconate-dehydrogenase (Boehringer-Mannheim). As a final step in the reaction series, NADPH is produced which can be visualised under long wave ultraviolet light. This electrophoretic system easily separates human galactokinase from mouse enzyme, as Fig. 1 shows.

All 23 hybrid clones selected in HAT medium contained the human chromosome 17 and displayed human galactokinase activity (Table 1). On the contrary, nine clones which were back-selected in medium containing BrdU and which have lost human chromosome 17, did not contain human galactokinase activity (Fig. 1 and Table 1).

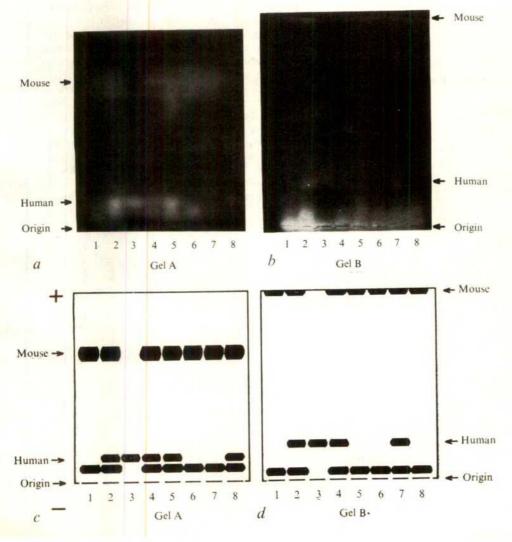


Fig. 1 Two different starch gels stained for galactokinase activity as before<sup>10</sup>, photographed under photographed under long wave ultraviolet light (a and b), c and d are diagrammatic representations of a and b respectively. Galactokinase extracted from mouse (Cl1D) and human (LNSV) parental cells each migrate as a single band of enzyme activity and are well separated in this electrophoretic system. Gel A was run at 8 V cm<sup>-1</sup> and gel B at 10 V cm-1 for 18 h at 4° C. demonstrates the wide degree of separation between human and mouse enzyme obtained in this system. All the hybrid clones tested were run with the higher voltage to obtain more effective of human galacseparation tokinase from the nonspecific staining that occurs near the origin in these gels when galactose deleted from the reaction mixture. Under these conditions mouse galactokinase migrates into the anode wick area of the gel. Gel A: channel 1, 52-58 clone 8 BrdU; 2, 52-58 clone 8; 3, LNSV (human parental cell line); 4, 52-58 clone 9; 5, 52-67 clone 21; 6, 52-67 clone 21 BrdU CIID (mouse parental cell line) 8, 52-58 clone 19. Gel B; channel 52-58 clone 8 BrdU; 52-58 clone 8; 3, LNSV; 4, 52-58 clone 9; 5, 52-58 clone 3 BrdU; 6, Cl1D; 7, 52-67 3 BrdU; 6, Cl1D; 7, 52-67 clone 21; 8, 52-67 clone 21 BrdU In gel A channels 1 and 2 and 5 and 6, and in gel B channels 1 and 2 and 7 and 8 represent pairs of HAT selected clones before and after BrdU selection.

These data demonstrate the syntenic relationship between human galactokinase and thymidine kinase and allow the assignment of the human gene for galactokinase to chromosome 17.

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## **Opossum Hb chain sequence** and neutral mutation theory

THE divergence of amino acid sequences which has accompanied evolution of species may be largely due to random fixation of selectively neutral or nearly neutral mutations. This was proposed by Kimura upon consideration of an inferred rapid rate of molecular evolution, the theory of the cost of natural selection, and observed high levels of polymorphism in natural populations. Among the observations considered to favour the neutral mutation-random fixation theory<sup>1-4</sup>, the apparent uniformity of the rate of fixation of amino acid substitutions in homologous proteins along several phyletic lines is outstanding. Kimura proposed this test of the neutral mutation theory by examining the amino acid sequences of proteins from so-called 'living fossils<sup>14</sup>. If, for a very long slowly evolving line, a rate of molecular evolution equal to that of more rapidly evolving lines is inferred (and it is assumed that the rate of evolution of physiological adaptations generally parallels the morphological evolutionary rate) then a case is made for the fixation of neutral mutations. The amino acid sequence proposed here<sup>5</sup> (Fig. 1) for the α chain of haemoglobin from a 'living fossil'6, Didelphis marsupialis, the Virginia opossum may be taken for such a test of the neutral mutation theory.

Since only a single haemoglobin component was found by gel electrophoresis7, chain separation8 was done on globin prepared from fresh haemolysate. Cyanogen bromide treatment's of the a chain yielded fragments containing residues 1-25, 26-76, 110-125, and 126-141; treatment with citraconic anhydride10, ethyleneimine11, and trypsin gave fragVal-Leu-Ser-Ala-Ash-Asp-Lys-Thr-Ash-Val-Lys-Gly-Ala-Try-Ser-Lys-Val-Gly-Gly-Asn-Ser-Gly-Ala-Tyr-Met-Gly-Glu-Ala-Leu-Tyr-Arg-Thr-Phe-Leu-Ser-Phe-Pro-Thr-Thr-Lys-Thr-Tyr-Phe-Pro-Asn-Tyr-Asp-Phe-Ser-Ala-Gly-Ser-Ala-Gln-Ile-Lys-Thr-Gln-Gly-Gln-Lys-Ile-Ala-Asp-Ala-Val-Gly-Leu-Ala-Val-Ala-His-Leu-Asp-Asp-Met-Pro-Thr-Ala-Leu-Ser-Ser-Leu-Ser-Asp-Leu-His-Ala-His-Glu-Leu-Lys-Val-Asp-Pro-Val-Asn-Phe-Lys-Phe-Leu-Cys-His-Asn-Val-Leu-Val-Thr-Met-Ala-Ala-His-Leu-Gly-Lys-Asp-Phe-Thr-Pro-Glu-Ile-His-Ala-Ser-Met-Asp-Lys-Phe-Leu-Ala-Ser-Val-Ser-Thr-Val-141 Leu-Thr-Ser-Lys-Tyr-Arg

Fig. 1 Proposed amino acid sequence for Didelphis marsupialis haemoglobin a chain.

ments containing residues 1-31, 32-102, and 103-141. Fragments were identified by compositions and sequences of tryptic peptides derived from them: identification of each tryptic peptide was generally inferred from obvious homology with a chains of known sequence. Identification of the peptide containing residues 57-61 was made by compositions of the parent fragments and identities of the other tryptic peptides derived from those fragments. Fragments and peptides were purified with columns of Sephadex or cation-exchange resins12. Sequence determination of the tryptic peptides was done by further enzymic or chemical cleavage and the subtractive Edman technique13. Amides were determined by aminopeptidase M digestion14 or glycinamidation15. (Full details of the structure determination will be published elsewhere.)

In Fig. 2 the number of sequence differences between members of several pairs of vertebrate  $\alpha$  chains<sup>18-24</sup> are listed. The opossum  $\alpha$  chain has apparently evolved more rapidly than any other a chain in all cases in which a comparison is possible. This result exceeds the expectations of the neutral mutation theory. A selectionist interpretation of the more rapid rate of molecular evolution in a living fossil is possible: if the evolutionary rate is limited by the cost of natural selection28-27 then one may expect to find an

	Cow	Horse	Dog	Rabbit	Man	Kangaroo	Opossum	Echidna	Chicken	Сагр
Mammalia Eutheria Cow <sup>16</sup> Horse <sup>17</sup> Dog <sup>18</sup> Rabbit <sup>19</sup> Man <sup>20</sup>	0	18	28 27 0	25 25 27 0	17 18 23 25 0	26 29 33 37 27	43 42 46 50 40	43 42 42 48 37	38 40 44 43 35	65 67 67 71 68
Metatheria Kangaroo Opossum	21					0	42 0	49 60	41 60	71 77
Prototheria Echidna <sup>22</sup>								0	48	75
Aves Chicken <sup>23</sup>									0	72
Osteichthyes Carp <sup>24</sup>	3									0

Fig. 2 Amino acid sequence differences between several pairs of vertebrate haemoglobin  $\alpha$  chains. Comparisons at sites involving the relative insertions or deletions in the carp sequence were not included.

especially keen rate of molecular evolution accompanying slow morphological changes because the reduced number of loci undergoing substitutions may sustain more intense selection per locus.

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### Preservation of antigenic properties of macromolecules over 70 Myr

It has been known for some time that many fossils still contain remnants of organic macromolecules, such as proteins and polysaccharides1. Such macromolecules are often enclosed inside the mineralised tissues of fossils, well protected from outside influences. This close association of skeletal, mineral and macromolecular substances, which is also found in recent mineralised tissues, suggests that this organic material has been an important factor in the process of mineralisation and has deeply influenced the physical properties of the skeletons<sup>2,3</sup>. It cannot be excluded however, that organic substances not involved in mineralisation can also be preserved in sedimentary rocks without much loss of their original structure, provided that they are well enclosed in the sediment. With this reserva-

tion in mind, characterisation of the structure of enclosed substances may yield valuable palaeobiochemical and diagenetical information.

Fossil macromolecules are often very complex, may occur in a great variety and may contain multiple decomposition products. Chemical analyses have hitherto been confined mainly to qualitative and quantitative determinations, such as amino acid analyses. In this paper we show that immunological techniques are very useful for the comparison of fossil and recent material from different sources. They may also be of value for the study of diagenetical processes.

We investigated cephalopods because fossil as well as recent representatives are readily available and contamination can easily be eliminated. Shells of the recent Sepia officinalis from the Dutch coast, of Nautilus pompilius (recent-Indian Ocean) and of Belemnitella junior (Maastrichtian-Maastricht, Netherlands) were scrupulously cleaned from organic and inorganic contamination and ground to a fine powder. Each powder was then decalcified in excess of 10% EDTA solution (pH 8) and insoluble residues were removed by centrifugation (30 min at 20,000g). The supernatant was concentrated and dialysed with an Amicon PM 10 filter until free of EDTA. One ml aliquots containing 1-2 mg material from Sepia and Belemnitella were injected with complete Freunds adjuvant into rabbits three times subcutaneously at intervals of two weeks. Against each antigen two antisera were prepared (AS, and ASI) against Sepia and AB1 and AB11 against Belemnitella). Extracts from the three different sources were compared in the Ouchterlony technique (Fig. 1). When the three extracts were allowed to react with preimmunisation sera, no precipitates were found. All the precipitates described are therefore immunoprecipitates.

Both Sepia antisera reacted with Sepia, Belemnitella and Nautilus extracts (Figs 1a and b). Depending on the antigen preparation (S<sub>1</sub> or S<sub>2</sub>) used, one or two precipitation lines were formed (Figs 1a and b). The AS11 serum indicates crossreactivity between Sepia and Belemnitella antigens, which is not shown with the AS<sub>1</sub> serum (Figs 1a and c). The AS<sub>11</sub> serum also detects crossreactivity between the Nautilus and Belemnitella antigens (Fig. 1d). Both AS sera produce very sharp lines with the Nautilus extract (Figs 1b and e). When tested with the AS<sub>1</sub> serum, no crossreactivity between the Sepia and Nautilus antigens is seen (Fig. 1e). This may be due to either the absence of the crossreacting antigen from the  $S_1$  antigen preparation (Figs 1a and b) or a crossreactivity between the Nautilus antigen and a hidden determinant of the Sepia antigens.

The experiments with the anti-Belemnitella sera produced only weakly visible patterns. From Fig. 1f it is clear, however, that single precipitation lines are formed with Belemnitella and Nautilus extracts indicating complete crossreactivity. The Sepia extract does not react with anti-Belemnitella serum, although the Belemnitella extract does react with the anti-Sepia sera (Figs 1a and c). This may indicate a weaker immunogenicity of the Belemnitella extract. From these experiments the following conclusions can be drawn. First, fossil as well as recent calcified tissues have been found to contain organic material with antigenic properties; second. because certain fractions from Belemnitella still crossreact with extracts of shells from recent Sepia (partially) identical structures have been retained in the fossil macromolecules for over 70 Myr. Third, the reactions of the Nautilus extract with the anti-Sepia and anti-Belemnitella sera suggest that the skeletal macromolecules in question have undergone minor alterations since the lower Carboniferous, when the Coleoids are thought to have diverged from the Nautiloid lineage. In evolutionary terms these must have been extremely conservative substances.

The antigenic relationship of the skeletal macromolecules of Cephalopods and related taxa is now being worked out in detail in our laboratory. The possibility of antigen localisation in sedimentary rocks by immunological techniques at light-

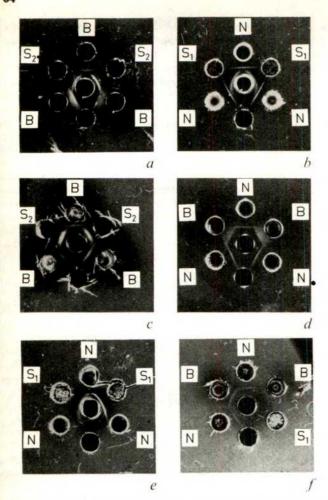


Fig. 1 Immunodiffusion patterns of extracts from shells of Sepia officinalis, Nautilus pompilius (both recent) and 70-Myrold Belemnitella junior. The central wells contained: anti-Sepia serum (AS<sub>1</sub>) (c and e); anti-Sepia serum (AS<sub>11</sub>) (a, b and d); Anti-Belemnitella serum (AB<sub>11</sub>) (f). B; Belemnitella extract; S<sub>1</sub> and S<sub>2</sub>, Sepia extracts; N, Nautilus extract.

and electron-microscopical level is also being investigated. Determination with immunological techniques of the phylogenetic relationship of taxa with unknown systematic position, such as the stromatoporoids, is yet another project which we intend to investigate in the near future.

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# Defective membrane synthesis in an *E. coli* mutant

THERE is a strict correlation between DNA synthesis and cell division1,2 but there is no known relationship between cell division and membrane synthesis-presumably because so little is known about the latter. We have previously reported that the formation of cytochrome  $b_1$  and the L- $\alpha$ -glycerophosphate uptake system occurs at the same time (in a discontinuous manner) at a particular phase in a cell cycle of Escherichia coli (ref. 3). On the basis of these observations we attempted to isolate a phase-specific mutant which has a defect in the formation of membrane components. The selection of the mutants was carried out assuming that cell growth is temperature-sensitive and that the formation of the β-galactoside transport system is under the control of the corresponding ts-mutation. These assumptions were based on the observation that the formation of the transport system for  $\beta$ -galactoside, measured using a synchronous culture, completely coincided with those of many membrane components including cytochrome b1 (M.O., unpublished) and on the consideration that cells defective for such processes should be non-viable4.

Here we present evidence that the mutant we isolated is unable to form cytochrome  $b_1$ , an  $L-\alpha$ -glycerophosphate transport system, an  $L-\alpha$ -glycerophosphate dehydrogenase and succinate dehydrogenase at a non-permissive temperature, because of a single mutational defect in a phase-specific function which operates 20–30 min before cell division. We also postulate two alternative functions of a protein which is synthesised or activated at 20–30 min before cell division. First, that the protein controls the fabrication of the active forms of membrane components, and, second, that the protein regulates a concurrent expression of the genes responsible for cytochrome  $b_1$  and so on, thus resulting in alignment of a population at a specific phase of cell division at a non-permissive temperature.

The mutant tsC42, described here, grows at a normal rate at 30° C but it stops growing at 42° C. Figure 1 shows that protein synthesis takes place at 42° C. The increase in the amount of protein began rapidly but gradually stopped. Finally, it reached a level of 1.7–1.8 times the original amount. The turbidity of the culture, the amount of protein and viable

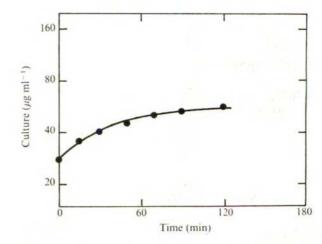


Fig. 1 Synthesis of protein in the mutant tsC42. Cells were grown in A medium<sup>5</sup> for several generations at 30°C and transferred to 42°C. Samples were withdrawn at the times indicated. Protein was measured by the method of Lowry et al.<sup>5</sup>.

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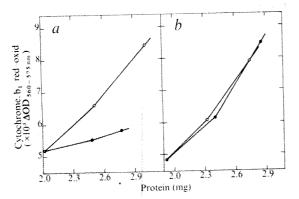


Fig. 2 Formation of cytochrome  $b_1$  in the mutant tsC42 and the parental strain AT713. Cells were grown in A medium<sup>5</sup> for more than three generations at 30° C and the culture divided into two parts. One portion was heated to 42° C and the other was further grown at the same temperature. Samples were removed after 20 and 40 min in the incubation at 42° C ( $\blacksquare$ ) and 30 and 60 min in the incubation at 30° C ( $\blacksquare$ ). The amount of cytochrome  $b_1$  was determined as described previously<sup>3</sup>. a, tsC42; b, AT713.

cells remained constant for a long period of incubation after initial increases. The size of the cells accumulated during incubation at a non-permissive temperature were homogeneous and almost equivalent to the larger cells of a cell population growing randomly.

Cytochrome  $b_1$  is the major component of cytochromes in  $E.\ coli$  and a membrane-bound constituent which is constitutively synthesised<sup>3</sup>. Figure 2 shows the results of experiments in which the formation of cytochrome  $b_1$  was measured in the mutant tsC42 and the parent AT713. The rate of formation was greatly reduced at  $42^{\circ}$  C, compared with  $30^{\circ}$  C in the mutant tsC42 (Fig. 2a), while their rates were essentially identical in the parent AT713 (Fig. 2b).

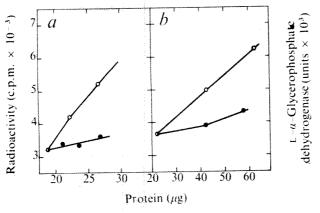


Fig. 3 Formation of L-α-glycerophosphate transport and L-α-glycerophosphate dehydrogenase in the mutant tsC42. Cells were grown in A medium<sup>5</sup> supplemented with 0.04 M DL-α-glycerophosphate. The assay of <sup>14</sup>C-L-α-glycerophosphate uptake in samples withdrawn after 30 and 60 min incubation at 30° C (○), and 20, 40 and 60 min at 42° C (♠), was carried out according to the method of Hayashi et al.<sup>7</sup>. For the assay of L-α-glycerophosphate dehydrogenase, samples were withdrawn after 30 and 60 min incubation at 30° C (○) and 20 and 40 min incubation at 42° C (♠) and the cells were washed twice with 0.1 M phosphate (pH 7.4) at 0° C and suspended in the same buffer. The cells were disrupted by treatment in a Umeda 20 kc 150 W sonic oscillator for 1 min and the unbroken cells were removed by centrifugation at a low speed. The enzymatic activity was assayed by measuring the rate of reduction of DCIP (2,6-dichlorophenol-indophenol) essentially according to the procedure of Lin et al.<sup>8</sup> except for the use of DCIP instead of MTT and measuring at 600 nm. The assay mixture contained: 0.30 ml cell-free extract, 2.0 ml 0.1 M phosphate at pH 7.4, 0.06 ml DCIP (1 mg ml<sup>-1</sup>), 0.30 ml phenazine methosulphate (1 mg ml<sup>-1</sup>), 0.20 ml 0.15 M KCN and 0.25 ml 0.4 M DL-α-glycerophosphate. One unit corresponds to 1 μmol DCIP reduced min<sup>-1</sup>. a, Transport of <sup>14</sup>C-L-α-glycerophosphate; b, L-α-glycerophosphate dehydrogenase.

Figure 3a shows the inability to form the transport system for L- $\alpha$ -glycerophosphate when the culture of the mutant was transferred into  $42^{\circ}$  C (after a full induction of the system by the supplement of 0.04 M DL- $\alpha$ -glycerophosphate). Since the transport once formed at a permissive temperature was fully active at  $42^{\circ}$  C, and as both the activities at  $30^{\circ}$  C and  $42^{\circ}$  C and the ratio of  $42^{\circ}$ :30° C are almost identical with those of the parental strain AT713, the inability of the inducer transport is not a cause for the absence of formation of the system at a non-permissive temperature. The inability of the mutant to form membrane components is also demonstrated for other enzymes. The results shown in Fig. 3b and 4 are for inducible membrane-bound L- $\alpha$ -glycerophosphate dehydrogenase and succinate dehydrogenase, being a typical and a constitutive membrane enzyme, respectively.

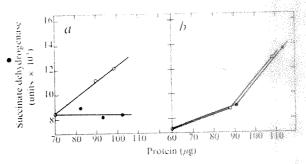


Fig. 4 Formation of succinate dehydrogenase in the mutant tsC42 and the parent AT713. The cultivation of cells and the preparation of the enzyme fraction are the same as described in the legend to Fig. 3 except that the induction by 1-a-glycerophosphate was omitted. The enzymatic activity was measured essentially according to the method described in the legend to Fig. 3. The assay mixture contained: 0.30 ml cell sonicate, 1.60 ml 0.1 M phosphate at pH 7.4, 0.06 ml DCIP (1 mg ml<sup>-1</sup>), 0.30 ml phenazine methosulphate (1 mg ml<sup>-1</sup>), 0.20 ml of 0.15 M KCN and 0.60 ml 0.5 M succinate. A unit corresponds to µmol of DCIP reduced min<sup>-1</sup>, a, tsC42; b, AT713.

The spontaneous reversion frequency to resistant cells at  $42^{\circ}$  C in tsC42 is between  $10^{-6}$  to  $10^{-7}$ ; within that known for single mutations. All three revertants examined were capable of forming cytochrome  $b_1$ , succinate dehydrogenase and L- $\alpha$ -glycerophosphate dehydrogenase at equal rates (increase of activity/increase of protein) at both  $30^{\circ}$  and  $42^{\circ}$  C, indicating that the pleiotrophic defect observed in the formation of several membrane components and cell growth was caused by a single mutation.

Figure 5 shows the patterns of cell division and the formation of succinate dehydrogenase when cells were transferred to 30° C after 35 min incubation at a restrictive temperature (42° C). Cell division occurred synchronously at 35 min and 135 min after a temperature shift to 30° C. The age of cells accumulated during the incubation at 42° C, deduced from the pattern of cell division, corresponds to 0.65 of a generation (designated as zero or one for cells at mid phase of cell division) and is compatible with the observed ages when many membrane components such as cytochrome  $b_1$  were formed during a synchronous growth3. The formation of succinate dehydrogenase occurred immediately after the shift from a non-permissive to a permissive temperature in a discontinuous manner. This result is consistent with the view that because of a defect in the regulation of membrane components formation the whole population of cells aligned at a phase 20-30 min before cell division during an incubation at a non-permissive temperature Furthermore, similar immediate formation was also observed in the case of the L- $\alpha$ -glycerophosphate transport system, thus supporting the above conclusion.

The doubling time (100 min) shown in this figure is comparable with one observed before the exposure to a non-permissive temperature. The synthetic activity of protein was restored promptly as soon as the culture was returned to 30° C. At essentially similar pattern of cell division was also obtained

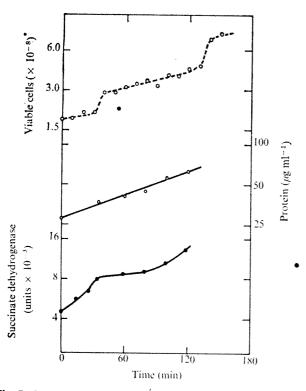


Fig. 5 Cell division and formation of succinate dehydrogenase after incubation at a non-permissive temperature in the mutant tsC42. Exponentially growing cells in A medium were shifted to 42° C and incubated for 35 min. Then the culture was returned to a permissive temperature (30° C) and the increases of viable cells (○---○) grown on a complete agar plate, succinate dehydrogenase (●) and total protein (○—○) were measured. Succinate dehydrogenase was measured according to the method described in the legend to Fig. 4 and units were expressed as µmol of DCIP reduced min<sup>-1</sup> ml<sup>-1</sup>. Total protein was measured according to the method in the legend to Fig. 1.

in the case of 90 min exposure to 42° C. These show that the exposure of cells to a non-permissive temperature had no vital influence and cells remained intact.

Our results indicate that either the conversion from inactive to active forms of the membrane components (or the coordinated expression of the genes responsible for cytochrome  $b_1$  and so on), is under the control of a protein, which is only synthesised or activated 20-30 min before cell division.

Bacterial membranes possess many important biochemical functions such as energy production, active transport, lipid synthesis and cell wall synthesis. They are also special sites for chromosome segregation. To fulfil such complex and ordered roles, membranes are fabricated in a highly organised structural form. The simultaneous formation of many membrane components under the same regulatory mechanism may be an obligatory requirement for the fabrication of membrane units possessing such complex structures from individual precursor molecules. A characteristic feature of the mutant tsC42 is that the mutation is pleiotrophic and the control for cell growth is strict. This means that the functioning or the synthesis of a phase-specific protein is concerned with cell division. Double labelling experiments which discriminate old membrane components from newly-formed ones show that the incorporation of a radioactive amino acid into membrane components was specifically restricted to some membrane components only in non-permissive conditions (M.O., unpublished). Our preliminary experiment presents further evidence for our tentative conclusion that membrane proteins essential for cell growth and cell division are under the strict control of a phase-specific protein with a regulatory function for the formation of membrane components.

Recently Jones and Donachie have reported that cell division is controlled by a protein which is synthesised between termination of chromosome rounds and cell division. Both our and their results show that phase-specific proteins play important roles in cell division.

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### Defect in the gamma polypeptide chain of a congenital abnormal fibrinogen (Paris I)

THE primary physiological function of fibringen is fibrin clot formation, a process that requires three distinct reactions. Thrombin first cleaves fibrinopeptides A and B from two of the three pairs of polypeptide chains  $(A\alpha, B\beta, \gamma)$  of fibrinogen<sup>1</sup>, following which the remaining fibrin monomers polymerise spontaneously into an insoluble fibrin clot<sup>2</sup>. Thrombin also converts Factor XIII to an active form, called plasma transglutaminase3, which in turn catalyses the formation of covalent  $\gamma$ -glutamyl- $\varepsilon$ -lysine isopeptide bonds<sup>4</sup> between two  $\gamma$  chains and more than two α chains of different fibrin molecules<sup>5</sup>. The resultant  $\gamma$  chain dimers and  $\alpha$  chain polymers crosslink the fibrin network and render it resistant to chemical or physical modifications.

Any of these three reactions could be impaired by structural defects of fibrinogen, but most of the heritable disorders are associated with either a decreased rate of fibrinopeptide cleavage by thrombin or defective polymerisation of fibrin monomers<sup>6,7</sup>. To date, a specific structural defect has been documented in only one abnormal fibrinogen, Fibrinogen Detroit<sup>8</sup>. The first well-studied abnormal fibrinogen, Fibrinogen Paris I9, showed a strikingly decreased ability to form fibrin polymers after reaction with thrombin and also inhibited the polymerisation rate of normal fibrin monomers. Studies of the fibrin monomers<sup>10</sup> suggested the presence of two populations of fibrinogen molecules in the propositus. Chemical analyses showed that fibrinopeptides A and B6, the NH2-terminal amino acids6, and the Aa chain of the NH2-terminal disulphide knot11 of Fibrinogen Paris I are normal. Here we describe experiments which demonstrate that Fibrinogen Paris I possesses an abnormal y chain, which is probably the cause of the impaired polymerisation of fibrin monomers. In addition, a second functional defect of this molecule will be documented, namely, its inability to crosslink in the presence of Factor XIII.

Fibrinogen (Fraction 1-0) was prepared by the technique of Blombäck and Blömback12 from normal citrated plasma and from citrated plasma of the propositus, in the presence of trypsin inhibitor (Iniprol, Choay, Paris). Factor XIII was obtained from fresh citrated bovine plasma according to the method of Loewy et al13. Human thrombin was supplied by Dr David L. Aronson, Bureau of Biologics, Food and Drug Administration, Rockville, Maryland.

The  $\alpha$  and  $\beta$  chains of non-crosslinked normal and Paris I

fibrin have the same electrophoretic mobility (Fig. 1, gels 1 and 2) and in both cases represent 30% and 36%, respectively, of the total protein, as calculated from densitometric scans (Densicord 552, Photovolt Co., New York). But, the band in the position of normal y chain stains much lighter in Paris I fibrin, and represents only 12% of the total protein compared with 34% in the normal fibrin. In addition, Paris I fibrin has an extra band between the positions of the normal  $\beta$  and  $\gamma$ chains representing 22% of the total protein. From this, Paris I fibrinogen probably contains two molecular populations of  $\gamma$  chain, of which about 35% is normal and about 65% is abnormal. The molecular weight of the abnormal variant of the γ chain is approximately 50,500 ±500, as determined from its migration in SDS-polyacrylamide gel, using standards of normal  $\alpha$ ,  $\beta$  and  $\gamma$  chains, which have molecular weights of 68,000, 56,000 and 48,000, respectively15.

The electrophoretic pattern of reduced normal crosslinked fibrin shows the expected absence of γ and α chains and the presence of  $\gamma$  chain dimers and  $\alpha$  chain polymers (Fig. 1, gel 3). Fibrin formed from Fibrinogen Paris I crosslinks only partially. The normal  $\gamma$  chain is absent and the  $\gamma$  dimer band is present (Fig. 1, gel 4). The abnormal γ<sub>Paris 1</sub> chain persists in the Factor XIII-treated fibrin, apparently not participating in the formation of γ-glutamyl-ε-lysine crosslink bonds. The concentration of a chain in Factor XIII-treated Paris I fibrin is less than in non-crosslinked fibrin. But 66%±15% of the α chains still remain in monomeric form, compatible with the small amount of a chain polymers present in Factor XIII-

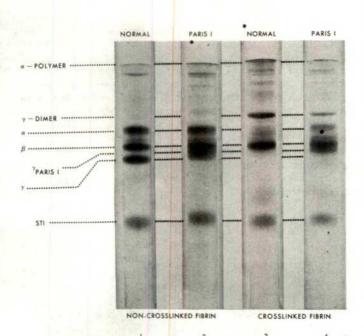


Fig. 1 The electrophoretic patterns in SDS-polyacrylamide gels of reduced fibrin samples obtained from purified normal human fibrinogen and Paris I fibrinogen. Non-crosslinked fibrin was obtained by adding 0.01 ml of human thrombin (100 NIH units ml-1) to a mixture of 0.1 ml fibrinogen (4 mg ml<sup>-1</sup>), 0.01 ml of soybean trypsin inhibitor (10 mg ml<sup>-1</sup>) (Sigma), 0.01 ml of freshly purified bovine Factor XIII (2 mg ml<sup>-1</sup>) and 0.01 ml of 0.1 M EDTA. Crosslinking of fibrin was accomplished by adding thrombin to a similar reaction mixture, except that 0.01 ml of 0.01 M calcium chloride was substituted for the EDTA. Reaction mixtures were incubated at 37° C for 24 h and then at 0° C for 1 h. After incubation, 0.015 ml of ethanol was added and the clots were dissolved by adding 0.5 ml of a solution containing 9 M urea, 3% sodium dodecyl sulphate and 3%  $\beta$ -mercaptoethanol and incubating at 37° C for 2 h. Aliquots of 5 µg of protein were subjected to SDS-polyacrylamide gel electrophoresis, performed according to the method of Weber and Osborn<sup>14</sup>, and gels were stained with Coomassie Brilliant Blue<sup>14,15</sup>. The rapidly migrating band present in the lower portion of each gel is soybean trypsin inhibitor (STI), which was added as an internal electrophoretic standard.

treated Paris I fibrin (Fig. 1, gel 4). The amount of a chain which does not participate in polymer formation correlates well with the proportion of γ chains that are abnormal (γ<sub>Baris 1</sub>). Since the a chains in Paris I fibrin are otherwise normal in appearance, this suggests that the failure of the abnormal YParis I chains to form dimers prevents the a chains of the same molecule from undergoing polymerisation. Thus, of the two varieties of fibrinogen in the plasma of the propositus, only those molecules with normal y chains undergo crosslinking of both the y and the a chains. This is supported by determination of the urea-soluble fibrin present in Factor XIII-treated Paris I fibrin. Overnight extraction with 5 M urea dissolves approximately 60% of the total fibrin, correlating well with the proportion of  $\gamma$  and  $\alpha$  chains in Paris I fibrin that does not crosslink. These changes are not the result of a Factor XIII defect, since this activity was normal in both the propositus and his father6.

The abnormal γ<sub>Paris I</sub> chains are about 2,500 daltons heavier than the normal y chains, corresponding to an additional polypeptide chain of about 22 amino acid residues. Since it is known that crosslinking of  $\gamma$  chains involves the glutamine and lysine residues in positions 14 and 6, respectively, from the carboxy-terminal end16, and since the NH2-terminal amino acids of Fibrinogen Paris I are the same as those of normal fibrinogen6, the molecular defect appears to involve the COOHterminal region. The heavier molecular weight of the Yparls I chain suggests that the structure is so altered as to impair the proper alignment of fibrin monomers for both polymerisation and Factor XIII-induced stabilisation. As such, this is the first documented example of an abnormal fibrinogen which

fails to crosslink. We thank Miss Margery E. Parker and Mrs Penny Shames for technical help. This work was supported by grants from the National Heart and Lung Institute. V.J.M. is the recipient of a Research Career Development Award from the National Heart and Lung Institute.

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### Carcinogenic polynuclear hydrocarbons bind to macromolecules in cultured human bronchi

THE ability of cells in human tissues to metabolise procarcinogens into oncogenic metabolites may be one important determinant in organ and host susceptibility to environmental carcinogens. Metabolic activation of polynuclear aromatic hydrocarbons (PNH) seems to be required before they can cause neoplastic transformation of cells either in vivo or in vitro1.2. This activation may involve the production of a metabolite(s) capable of tight binding to cellular macromolecules including DNA. The binding level of PNH to macromolecules generally shows a good correlation with their oncogenicity in experimental animals and cells in culture3-6, although exceptions exist7. Whether or not this correlation exists in man is unknown.

We report here that human bronchial epithelial cells in organ culture have the capability to bind and presumably to activate PNH [7.12-dimethyl benz(a)anthracene (DMBA): 3-methylcholanthrene (MCA); benzo(a)pyrene (BP) and dibenz(a,h)anthracene (DBA)]. Binding to DNA was measured by biochemical methods while total binding to epithelial cell macromolecules was shown by autoradiography. These methodologies had been previously developed in studies utilising an animal model of human lung

Bronchi were obtained as surgical specimens from three patients with lung cancer and from one patient without lung cancer. Grossly normal-appearing pieces of bronchi were immediately immersed in L15 medium10 at 4°C until cultured. The bronchi were then cut into approximately 1×1 cm squares and placed in 60 mm plastic Petri dishes with the epithelium oriented toward the gas-liquid interface. Two ml of CMRL 1066 medium<sup>11</sup> containing 1 µg ml<sup>-1</sup> crystalline insulin, 0.1 µg ml<sup>-1</sup> hydrocortisone hemisuccinate, 0.1 μg ml<sup>-1</sup> β-retinyl acetate, 100 units ml<sup>-1</sup> penicillin G, 2 mM L-glutamine and 100 µg ml<sup>-1</sup> streptomycin were added to each dish17. Cultures were maintained in an air-tight box and gassed with 50% O2, 45% N2 and 5% CO2 for 5 min. The medium was replaced and the cultures were gassed every 2 d. The box containing the cultures was rocked in the dark at approximately 10 cycles min-1 so that the bronchial tissue was submerged half of the time. Cultures were incubated at 36.5° C.

At 7 d, tritium labelled PNH (3H-DMBA, 14.5 Ci mmol-1, (Amersham/Searle,); <sup>3</sup>H-MCA, <sup>5</sup> Ci mmol<sup>-1</sup> (Amersham/Searle); <sup>3</sup>H-BP, <sup>17</sup> Ci mmol<sup>-1</sup>, (Amersham/Searle) and <sup>3</sup>H-DBA, <sup>2</sup>42 Ci mmol<sup>-1</sup> (New England Nuclear)) were dissolved in dimethyl sulphoxide (final concentration 0.5%) and added to the culture medium at a final concentration of 40 µCi ml<sup>-1</sup>. The radiopurity of these compounds was determined by radioscan of thin layer chromatograms. When necessary the compounds were repurified by chromatography8 to a radiopurity of greater that 99%. Following incubation for 24 h, the bronchial tissues were washed three times with cold Dulbecco's phosphate-buffered saline. For autoradiography four cultures per experimental variable were fixed overnight in 3% glutaraldehyde buffered by 0.1 M s-collidine, pH 7.4, and then postfixed in 1.33% OsO4 in the same buffer. As

Table 1 Specific activities of binding of tritium-labelled polynuclear hydrocarbons to human bronchial DNA

Polynuclear	Specific activity*						
hydrocarbon	(d.p.m. per µg DNA)	(pmol per mg DNA)					
DMBA	170 ± 22† (3)‡	53+7					
BP	224 + 77 (4)	$40 \pm 14$					
MCA	$38\pm 9$ (2)	34+8					
DBA	$15\pm 3$ (3)	28±6					

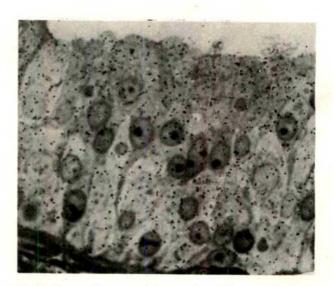
\* The amount of DNA and d.p.m. were determined from the peak DNA fraction of CsCl gradients. See text for specific activities (Ci mmol<sup>-1</sup>) of tritiated PNH.

†Mean ± s. e. ‡Number of cases studied.

previously described, tissues were repeatedly washed in dehydrating solutions of ethanol to remove unbound PNH; The radioactivity released into the dehydrating solutions was monitored by liquid scintillation methods. The tissues were then embedded in Epon 812 containing 5% Araldite 502, 1 μm sections were cut, and autoradiograms were prepared12

Radioactivity was found in both the nuclei and cytoplasm of epithelial cells incubated with 3H-DMBA (Fig. 1). Ciliated, mucous and basal cells contained tightly bound radioactivity. Epithelial cells in bronchial glands were overlaid also with autoradiographic grains. Similar results were found with 3H-BP, 3H-MCA and 3H-DBA. Quantitative analysis of the autoradiograms will be reported fully elsewhere. Bound radioactivity was also found in mesenchymal cells, but to a lesser extent. This observation is consistent with the finding that human embryonic lung fibroblasts are capable of activation and binding of PNH in vitro13. For biochemical studies, bronchial mucosa cells were scraped from the supporting cartilage. Cells from six cultures were pooled for each carcinogen-case combination. DNA was isolated from these cells, and the results of CsCl gradients of the purified DNA are shown in Fig. 2 and Table 1. In the cases studied to date, DMBA and BP bind to DNA more than DBA.

We wished to determine if a major target tissue of environmental carcinogens, the human bronchus, had the capability to activate PNH into forms which tightly bind to macromolecules. It is apparent that the human bronchial mucosa has this capability after it has been maintained



Autoradiogram of human bronchial epithelial cells following in vitro incubation with 3H-DMBA (40 μCi ml-1) for 24 h. Autoradiographic grains are found overlying both nuclear and cytoplasmic sites. One micrometre section stained with toluidine blue (×880).

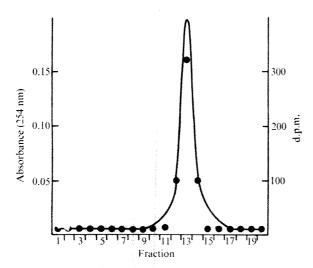


Fig. 2 CsCl gradient profile of DNA purified from human bronchial mucosa. After 7 d in organ culture, bronchial pieces were incubated with  ${}^{3}\text{H-MCA}$  (40  $\mu\text{Ci ml}^{-1}$ ) for 24 h. The bronchial mucosa containing epithelial and mesenchymal cells was then scraped from supporting structures and a crude preparation of DNA was obtained by phenol extraction and precipitation with 50% ethanol. The precipitate was repeatedly extracted with ether and benzene to remove unbound tritiated PNH and sequentially digested with RNase (Worthington Biochemicals) and Pronase (Calbiochem). The aqueous solution of DNA was extensively (Worthington Biochemicals) and Pronase dialysed and then banded on CsCl gradients (Beckman SW56 rotor, 35,000 r.p.m.; 66 h, 25° C). Gradients were fractionated into 0.2 ml portions, and absorbance and radioactivity were determined on each fraction. The absorbance (—) was continuously monitored at 254 nm; radioactivity (d.p.m.) is indicated by the points. Each gradient contained approximately 10 µg of DNA.

in organ culture for 7 d. This finding is consistent with previous studies<sup>14,15</sup> showing morphological changes in human respiratory epithelium caused by PNH in vitro.

As this system becomes better defined, it may be used to determine the individual variations of binding activity in groups at different risks to bronchogenic carcinoma; in adition this system may be useful in evaluating experimental attempts to modify and inhibit the activation of PNH in the human bronchial epithelium. Studies comparing the level of PNH binding to DNA in a large number of individuals with and without lung cancer are needed. If groups at high risk to develop lung cancer can be identified then steps to intervene can be taken<sup>16</sup>. We thank William Kaufmann, Frank Jackson and Maria Yamaguchi for technical assistance. The comments of Drs Yuspa, Sporn, Saffiotti and Janss were appreciated by the authors. This work was supported in part by a National Cancer Institute contract.

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### Dose-related inhibition of chemical carcinogenesis in mouse skin by caffeine

THERE is some evidence that caffeine protects against the effects of chemical carcinogens<sup>1,2</sup>. Leitner and Shear<sup>3</sup> found a reduction in the effectiveness of benzo(a)pyrene as a carcinogen when it was administered by subcutaneous injection together with various purines, including caffeine. An enhancement of the antitumour effect of 1,3-bis(2-chloroethyl)-1nitrosourea by caffeine has been reported4 but whether the caffeine acts as an anticarcinogen or as a membrane-active agent (a property of caffeine which has been demonstrateds) is not clear. Tumour production in vivo has been shown to be partially suppressed by theophylline<sup>6</sup> and it is possible that caffeine which is chemically closely related to theophylline may similarly suppress tumour production.

We have shown that caffeine inhibits the carcinogenic action of cigarette smoke condensate fractions when added to the fractions before application. Our experiments were prompted by unexpected results obtained when testing fractions of cigarette smoke condensate for carcinogenicity. Traces of caffeine, remaining after a fractionation procedure involving caffeine7, were found in fractions which showed reduced carcinogenicity when applied to mouse skin. The effect of caffeine was then tested systematically.

Two fractions of smoke condensate, from plain cigarettes manufactured from flue-cured tobacco representing the major plain cigarette brands smoked in the United Kingdom, were prepared by standard methods7-9 and applied repeatedly to mouse skin at two dosages either alone or together with caffeine, also at two concentrations. Dose levels of condensate fractions refer to the amount of smoke condensate from which the fraction was derived; thus an application of 150 mg of a fraction which represented 25% by weight of the parent condensate is referred to as a 600 mg dose. All treatments were given as solutions/suspensions in 0.3 ml of isopropanol: acetone (20:80) three times weekly until the last mouse died (112 weeks). Procedures used for animal husbandry, skin shaving, the application of fractions, post mortem and histopathological examination of mouse skin were as previously described9,10

The two fractions tested were: first, fraction G, a cyclohexane soluble fraction, prepared from smoke condensate by removal of water soluble materials followed by distribution of the water insoluble phase between 90% methanol and cyclohexane<sup>8</sup>. This fraction, representing 25% by weight of the original condensate, accounted for 80% of the carcinogenic activity of the condensate. Second, fraction RP prepared by extracting a cyclohexane solution of fraction G with a 15% w/v solution of caffeine in 90% formic acid followed by removal of the caffeine?. This fraction, representing 3.3% by weight of

Table 1 Effect of topically applied caffeine on the incidence of skin tumours in mice in response to fractions of cigarette smoke condensate applied to the skin

			appined t	o the skill				
	Treatment app	olied thrice week	ly	<b>3.</b> 7 .				
	Actual dose per	Equivalent	Dose of	No. of	l ui	nour-bearing animals	In	filtrating-carcinoma bearing animals
Condensate	application	dose of condensate	caffeine per application	animals per	R	elative incidence rate $b \times 10^6$		Relative incidence
fraction G	(mg) 2 <b>5</b>	(mg) 100	(mg)	group		=3, w=15.26)	%	rate $b \times 10^{12}$ ( $k = 6, w = 12.55$ )
G G	50 25	200	ő	51 51	37 61	2.4 8.5	14 35	1.3 4.3
Ğ	50	100 200	0.04 0.08	51 51	57 67	3.9 6.7	14 20	1.4
G G	25 50	100 200	0.20 0.40	51	41	2.3	12	2.6 0.9
RP RP	3.3	100	0.40	51 51	47 51	3.6 3.7	20 18	2.3 1.8
RP	6.6 3.3	200 100	0 0.04	51 51	75 24	7.3 1.6	31	4.1
RP RP	6.6 3.3	200 100	0.08 0.20	51	61	5.8	28	1.0 5.0
RP Caffeine	6.6	200	0.40	51 51	37 47	2.4 3.6	10 16	1.0 1.7
Caffeine	0		0.08 0.40	51 51	0	0.0 0.1	0	0.0
Untreated control Solvent control	0		0 •	102	2	0.1	0	0.0 0.0
	-		0	102	2	0.1	0	0.0

the original smoke condensate, accounted for more or less all the carcinogenic activity of the parent fraction, G. The concentration of caffeine added to the mouse painting solutions were: 0.04% of the smoke condensate equivalent (that is,  $0.24~\mathrm{mg~week^{-1}}$  for a 600 mg dose and  $0.12~\mathrm{mg~week^{-1}}$  for a 300 mg dose) or 0.2% of the smoke condensate equivalent (that is 1.2 mg week<sup>-1</sup> for a 600 mg dose and 0.6 mg week<sup>-1</sup> for a 300 mg dose). Caffeine was also tested alone at doses of 0.24 mg week -1 and 1.2 mg week -1.

The results, given in Table 1, are presented as percentage of animals bearing one or more benign or malignant tumours (TBA), percentage of animals bearing carcinomas showing invasion of the panniculus muscle on microscopic examination (CBA) and the relative incidence rates for tumours and carcinomas.

The relative incidence rate parameter, b, is obtained by the fit of a Weibull distribution which postulates that the incidence rate at time t, in treatment group i, is given by the expression  $b_i k(t-w)^{k-1}$  in which k and w are independent of the treatment<sup>11</sup>. The relative incidence rate is a better measure of effect than are simple percentages as it compensates for different mortality rates in the groups.

From the results it is clear that caffeine alone did not lead to the development of skin tumours. To evaluate the effect of the three variables (fraction, dose and caffeine) in the other groups, the method of Weibull multiple regression was used12. The analysis showed that there was a highly significantly greater response (P<0.001 for both TBA and CBA) at 600 mg dose levels than at 300 mg dose levels. In fact the response at 600 mg was, on average, 2.2 times greater than at 300 mg for tumours and was 2.6 times greater for carcinomas. At the higher caffeine concentration, the caffeine significantly reduced tumour incidence (P<0.001 for TBA and <0.005 for CBA). At the lower caffeine concentration, there was also a reduction in tumour incidence but the effect was not statistically significant. There was no evidence of a difference in response between the two fractions, nor of any interaction between the three factors: fraction, dose of fraction and concentration of caffeine.

Although it is not known how caffeine brings about these effects, it is known that purines form complexes with polycyclic aromatic hydrocarbons (PArH) and heteropolycyclic compounds. Both these chemical classes have representative compounds in cigarette smoke condensate and some members of both classes are chemical carcinogens. The complexing is relatively weak in the case of the PArH; resulting from a dipole-induced dipole interaction between the polar component (caffeine) and the polarisable component (the aromatic compound).

Attempts have been made to relate complexing ability of PArH to their carcinogenic properties, but none has been successful1,2. It is nevertheless possible that the complexing property of PArH makes an important contribution to their carcinogenicity, the differences in their activity resulting from a combination of factors such as molecular size, geometry and general reactivity. Thus, the absence of carcinogenic activity in the majority of PArH might arise from diffusion and transport difficulties within the cell or a lack of ability to adapt to a favourable configuration at the site of action within the cell. The inhibition of carcinogenesis by caffeine could result from competition for the polycyclic compound between the site of action in the cell and the caffeine.

The possibility that caffeine may inhibit transport of PArH through cell membranes cannot be overlooked, but caffeine can facilitate the transport of some compounds through membranes, so this explanation seems unlikely.

Both caffeine and theophylline are known to affect the level of adenosine 3', 5'-cyclic monophosphate (cyclic AMP) in mammalian cells13. It has been suggested that a partial suppression of tumour production by theophylline was due to an effect on cyclic AMP synthesis6 and caffeine may inhibit tumour production by a similar mechanism. If this were the correct explanation, caffeine could be expected to act as an inhibitor of chemical carcinogenesis in general and not specifically of polycyclic hydrocarbon induced carcinogenesis. This possibility can therefore be tested.

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### Caffeine inhibits induction of endogenous C-type virus

Endogenous C-type viruses can be induced by treating the rodent cells in which they reside with halogenated pyrimiding analogues, notably 5-iodo-2'-deoxyuridine (IUdR) (refs 1, 2). Here I show that caffeine, which inhibits repair of photolesions and interacts with DNA in various ways<sup>3</sup>, inhibits the induction of the endogenous virus by IUdR.

The MLg cell line originating from a newborn ddY mouse was used; this cell line was found to release C-type virus after IUdR treatment'. The induced virus was detected by the XC test5.

The frequency of virus production was estimated as follows. MLg cells inoculated in 60 mm plastic Petri dishes (10<sup>s</sup> cells per dish) were treated with IUdR (5-50 µg ml<sup>-1</sup>) for 24 h; the medium was removed and untreated MLg cells (103 cells per dish) were laid over the IUdR-treated cells. Three days later, three subcultures were made; after a further 3 d, the cultures were submitted to the XC plaque titration test. For IUdR concentrations of 5, 20 and 50 μg ml<sup>-1</sup> the mean plaque number per dish was 19, 73 and 125, respectively, A proportional relation between plaque number and IUdR concentration was observed. There was some variation of induction frequency from one experiment to another, but the variation was small within one experiment, (see Fig. 1a). In the subsequent experiments, the concentration of IUdR was used at 25 µg ml<sup>-1</sup> for 24 h.

The cells were treated with caffeine (0.625 to 5 mM) for 24 h before or after IUdR treatment (25, µg ml<sup>-1</sup> for 24 h). Twenty-four hours after the removal of IUdR, the cultures were overlaid with normal MLg cells (10<sup>5</sup> cells per dish). Three days later, each culture was divided into three, and

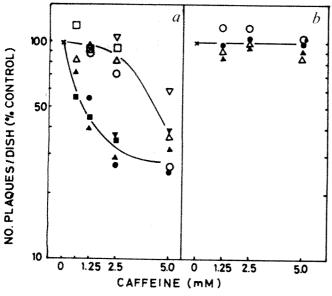


Fig. 1 a, Effect of caffeine on endogenous C-type virus induction. Closed symbols indicate caffeine treatment after IUdR (2 ml-1), and open symbols indicate caffeine treatment before IUdR. Four repeated experiments are shown. The mean number of plaques per dish was obtained from three cultures which were derived from one plate. In some experiments, 2 cultures were prepared for the control; the mean plaque number in this case is the mean of the two values obtained. The mean plaque number of the control was 129 for experiment  $1(\bigcirc, \bigoplus)$ , 134 and 119 for experiment  $2(\triangle, \triangle)$ , 114 and 98 for experiment 3 ( $\square$ ,  $\blacksquare$ ), and 95 and 85 for experiment  $4(\nabla, \blacktriangledown)$ . b. Effect of caffeine on exogenous Friend leukaemia virus infection. Open symbols indicate treatment before infection and closed symbols after infection. Duplicate experiments are shown. Three dishes were used for each point. The mean plaque number in the control dishes was 125 and 109 for experiment 1 (○, ●) and experiment 2 (△, ▲), respectively.

submitted to the XC test. Figure 1a shows that caffeine treatment after IUdR treatment significantly inhibited virus induction; a proportional relation was observed between caffeine concentration and the inhibition of induction of the C-type virus. Treatment with doses of caffeine as high as 2.5 mM before IUdR treatment was almost without effect.

In order to test the effect of caffeine on the exogenous C-type virus infection, MLg cells were treated with caffeine (1.25 to 5 mM) for 24 h before or after infection with Friend murine leukaemia virus (adsorption for 2 h). Twenty-four hours after infection, 10<sup>3</sup> normal MLg cells were added per dish. Three days later, the XC test was performed. Figure 1b shows that caffeine was without effect on exogenous virus infection.

Various ways of inhibiting cellular DNA synthesis, such as treatment with arabinosyl cytosine, excess thymidine and serum starvation, have been reported to inhibit virus induction<sup>6</sup>. The inhibition was presumably due to inhibition of •IUdR incorporation. These conditions also inhibit exogenous C-type infection7,8; consequently, in some cases, propagation of the induced virus may be inhibited.

Since caffeine affects neither the exogenous virus infection nor incorporation of 128 IUdR (data not shown) caffeine may interfere with events specific to virus induction.

What happens between IUdR incorporation into the cellular DNA and the subsequent release of the induced virus is obscure. Excision of the viral genome may or may not be necessary. In any event, viral induction may be brought about by some toxic modifications of DNA by IUdR. It is possible that IUdR may induce local distortion of DNA as the halogenated pyrimidines when incorporated into DNA cause fragmentation or instability of DNA. Caffeine, known to interact with denatured but not with helical DNA<sup>9</sup>, may interact with such distorted regions of the DNA, so as to prevent viral gene activation. This last suggestion gains support from the fact that caffeine was especially effective after IUdR incorporation.

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### Isolation of an inhibitor protein of E. coli RNA polymerase from T7 phage infected cell

BACTERIOPHAGE T7 gene expression is under a unique transcriptional control. The 'early to late' switch in T7 development involves a switch from the host Escherichia coli RNA polymerase to the T7-coded enzyme (T7-specific RNA polymerase1), transcribing early mRNA and late mRNA, respectively, from two different regions of the T7 genome<sup>2</sup>. In addition to the gene 1 coding for T7-specific

RNA polymerase, this early-late switch requires the function of another early gene of T7, gene 0.7 or the 'host shut-off' gene<sup>2,3</sup>, to shut off the synthesis of host RNA and T7 early mRNA. This shut-off function is assumed to be an inactivation of the host RNA polymerase, although this has not been proven<sup>4,3</sup>. Furthermore, we have previously shown that T7 early mRNAs are functionally unstable, although they remain chemically stable late in T7 infection<sup>6,7</sup>. Thus, it seems that a complete switch of T7 gene expression from early to late involves at least three different control mechanisms. It has been shown that a T7-coded translational repressor may also play a role in the elimination of host functions<sup>6</sup>.

Here we report that the 'host shut-off' function is indeed an inactivation of the host RNA polymerase molecule, involving an inhibitor protein bound to the enzyme. When this inhibitor protein, here referred to as 'I' protein, is removed from the inactive host RNA polymerase prepared from T7 infected cells, the core enzyme and the sigma factor are both functionally active. The I protein, with a molecular weight of about 66,000, has been purified. It inhibits the host RNA polymerase holoenzyme, but does not inhibit the core enzyme or the T7-specific RNA polymerase.

Figure 1 shows the switch from the host RNA polymerase to the T7-specific enzyme in normal T7 infection. Cell-free extracts were prepared from T7 infected cells at the times indicated and assayed for the two enzymes. A rapid reduction of the host RNA polymerase activity coincided with a rapid increase of the T7-specific RNA polymerase activity. Since addition of rifampicin 5 min or later after T7 infection did not change the kinetics of appearance or the final yield of T7-specific RNA poly-

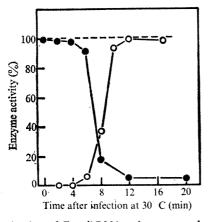


Fig. 1 Inactivation of E. coli RNA polymerase and appearance of T7-specific RNA polymerase after T7 infection. E. coli D10 F- (ref. 6) was infected with T7 phage at a multiplicity of infection of 5 at 30° C. Cells were also infected with T7 in the presence of chloramphenicol (150 µg ml<sup>-1</sup>) added 5 min before infection. Aliquots of the culture were withdrawn at indicated times and the cells were collected by centrifugation. The cells were suspended in a buffer containing 0.05 M Tris-HCl, pH 7.9, 0.01 M MgCl<sub>2</sub>, 0.15 M KCl, 1 mM dithiothreitol (DTT) and 0.1 mM EDTA and sonicated. Sonicated extracts were centrifuged at 12,000g for 10 min. Approximately 400 µg (protein) of the cell-free extract was used for the assay of *E. coli* RNA polymerase activity. The standard assay mixture<sup>19</sup> contained 5 µg T4 DNA in a total volume of 0.25 ml and was incubated at 37° C for 15 min. The sonicated cell-free extract was also used for the assay of T7-specific RNA polymerase activity following the method previously described<sup>1,7</sup>. The assay mixture contained 5.6 µg T7 DNA and 5 µg rifampicin in a total volume of 0.25 ml, and was incubated at 37° C for 10 min. Since rifampicin inhibits only E. coli RNA polymerase, this assay is specific for T7-specific RNA polymerase. Enzyme activities were normalised by measuring the protein content of each extract. The 100% values were: RNA polymerase, (--), E. coli RNA polymerase in the presence of chloramphenicol; (()), T7-specific RNA polymerase.

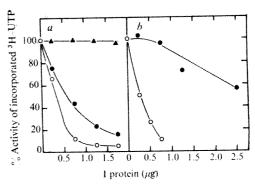


Fig. 2 Specific inhibition of *E. coli* RNA polymerase holoenzyme by I protein. *a*, Standard RNA-synthesising system<sup>19</sup> contained <sup>3</sup>H-UTP and 2.4 μg T4 DNA or 4.4 μg poly(dAT), and was incubated at 37° C for 15 min. Various amounts of purified I protein, electrophoretically more than 90% pure, were added to the reaction.  $\bigcirc$ , *E. coli* RNA polymerase holoenzyme (4.4 μg) on T4 DNA. The 100% value was 23,000 c.p.m. per reaction.  $\bigcirc$ , *E. coli* RNA polymerase holoenzyme (4.4 μg) on poly(dAT). The 100% value was 35,000 c.p.m. per reaction.  $\bigcirc$ , *E. coli* RNA polymerase core enzyme (3 μg) on poly(dAT). The 100% value was 45,000 c.p.m. per reaction. *b*, *E. coli* RNA polymerase holoenzyme was assayed as in *a* with 4.5 μg T7 DNA. T7-specific RNA polymerase was assayed as in Fig. 1, with 4.5 μg T7 DNA in the presence of rifampicin<sup>1-7</sup>. The actual molar amount of T7-specific RNA polymerase protein used was about one-twentieth of that of *E. coli* RNA polymerase. Increasing amounts of I protein were added. ( $\bigcirc$ ), *E. coli* RNA polymerase on T7 DNA. The 100% value was 26,000 c.p.m. per reaction. ( $\bigcirc$ ), T7-specific RNA polymerase on T7 DNA. The 100% value was 27,000 c.p.m. per reaction.

merase<sup>7</sup>, we assume that no new initiations of T7 early mRNA transcription by the host RNA polymerase occur after this time (T7-specific RNA polymerase is one of the early gene products<sup>2</sup>). This shut-off of T7 early mRNA synthesis, probably concomitant with the shut-off of host RNA synthesis, parallels with the decline of the host RNA polymerase activity (Fig. 1). When the cells were infected with T7 in the presence of chloramphenicol, no reduction in the activity of the host RNA polymerase was observed, indicating that protein synthesis after T7 infection is required to accomplish the shut-off function.

The result shown in Table 1 was obtained after removing endogenous DNA from sonicated cell-free extracts by DNase treatment. DNase-treated extracts were centrifuged in glycerol gradients and the lower portions of the gradients, containing the host RNA polymerase activity but free of DNase activity, were pooled and assayed for RNA synthesis on T4 phage DNA and poly(dAT) templates. The crude enzyme from uninfected cells exhibited an activity of above 1.2 on T4 DNA relative to that on poly(dAT) and this activity ratio, T4 DNA: poly(dAT), reached about 2.0 after purification. The crude host enzyme from T7 infected cells however, showed an activity ratio of only about 0.2. A considerable activity on poly(dAT) in the enzyme from T7 infected cells was used to monitor its recovery during purification. The presence of chloramphenicol prevented the reduction of the host RNA polymerase activity in T7 infected cells (Table 1).

The E. coli RNA polymerase was purified from uninfected control cells and T7 infected cells harvested 9 min after infection, using essentially the same procedure described by Burgess. After the first glycerol gradient purification step in the absence of KCl, the control enzyme showed an activity ratio on T4 DNA against poly(dAT) of between 1.5 and 2.0, whereas the enzyme from T7 infected cells showed a ratio of about 0.2. After the second glycerol gradient step in the presence of 1 M KCl, the relative activity on T4 DNA and poly(dAT) of the control enzyme was about 2.0, while the enzyme from T7 infected cells displayed a value about 1.0. The increased activity of the enzyme from T7 infected cells on T4 DNA after

the second gradient step was due to a partial removal of inhibitory material. When gradient fractions sedimenting more slowly than the enzyme were added back to it, its relative activity on T4 DNA and poly(dAT) was about 0.2, the same as that before the second gradient step.

The purified enzymes, after the second gradient step, were analysed by electrophoresis in polyacrylamide-SDS slab gels. The enzyme from T7 infected cells contained, in addition to  $\alpha$ ,  $\beta$ ,  $\beta'$  subunits and sigma factor, two protein components which were usually absent in the control enzyme and when present, only in a trace amount. One protein component tentatively termed as I has been purified as described below, and found to be an ihibitor of the host RNA polymerase. The other protein migrating slightly faster than the I protein has also been purified, but has no effect on the RNA polymerase activity.

The RNA polymerase from T7 infected cells recovered full sigma and core enzyme activities when the inhibitor protein I was completely removed. This was shown by subjecting the host RNA polymerase from T7 infected cells to phosphocellulose column chromatography. The protein I was separated from the enzyme complex and eluted in the flow-through. The sigma factor and core enzyme were also separated. Sigma factor was further purified by DEAE column chromatography and glycerol gradient centrifugation. The purified sigma factor and the core enzyme eluted from the phosphocellulose were electrophoretically more than 95% pure. Reconstitution experiments showed that purified sigma factor and core enzyme from T7 infected cells are functionally normal in forming fully active RNA polymerase holoenzyme both with each other, and with samples purified from uninfected cells.

The I protein was purified from the flow-through of the phosphocellulose column and by DEAE cellulose column chromatography followed by glycerol gradient centrifugation. Purified I protein moved slightly faster than bovine serum albumin in electrophoresis in the presence of SDS and its molecular weight was estimated as about 66,000. In some cases it was purified from the enzyme preparation after the first glycerol gradient, because at this step the enzyme contained more I protein than after the second

Figure 2a shows the inhibition of E. coli RNA polymerase holoenzyme by purified I protein. RNA synthesis on T4 DNA was inhibited almost completely at a molar ratio of I to enzyme of about 1. The holoenzyme acivity on poly(dAT) was also inhibited by I, but to a slightly lesser extent. No inhibition was observed when I protein was added to the core enzyme synthesising RNA on poly(dAT). Figure 2b also shows the inhibition of the holoenzyme by purified I protein in T7 DNA-directed RNA synthesis. At a concentration of I protein (0.75  $\mu$ g) which inhibited the host RNA polymerase by 90%, T7-specific RNA polymerase was not inhibited. At higher I protein concentrations, T7-specific RNA polymerase was somewhat inhibited. Because the molar amount of T7-specific RNA polymerase used in the reaction was about one-twentieth of that of the host enzyme, however, at these concentrations of I protein the ratio of I to the T7-specific RNA polymerase was very high.

These results indicate that the inhibition by I protein is specific for the host RNA polymerase holoenzyme and that the presence of sigma factor is required for this inhibition. The details of the interaction between I protein and the holoenzyme are under investigation. Since the core RNA polymerase of the host and the T7-specific RNA polymerase both synthesised normal amounts of RNA in the presence of I protein, at a concentration which effectively inhibited RNA synthesis by the host holoenzyme on the same templates (Fig. 2), I protein does not seem to have DNase or RNase activity. Also, T7 DNA-directed RNA synthesis by the host holoenzyme was inhibited by I protein

to a similar extent as shown in Fig. 2b even when four times more T7 DNA was added as template. This result suggests that DNase activity in I protein is unlikely and supports the idea that I protein interacts with the holoenzyme rather than with the DNA template.

We have not yet determined whether I protein is directly coded by T7, or is instead a host protein somehow activated or induced by T7 infection. It is certain, however, that protein synthesis after T7 infection is required to manifest the inactivation of the host RNA polymerase by I protein, since the presence of chloramphenical permits the host RNA polymerase to remain fully active (Fig. 1, Table 1). If I protein is coded by T7, the most likely

Table 1 E. coli RNA polymerase activity after T7 infection

Conditions of T7 infection	Activity on T4 DNA (%)	Activity on poly(dAT)	Ratio* T4 DNA poly(dAT)
Uninfected 9 min after infection	100† 17	100† 43	1.271 0.21
9 min after infection, (With chloramphenicol)	98	specific to	

Sonicated cell-free extracts, prepared as in Fig. 1, were treated with 150 µg ml<sup>-1</sup> DNase at 0° C for 15 min and centrifuged at 20,000g for 15 min. The extract was layered on a 15-30% glycerol gradient in 12 ml containing 0.01 M Tris-HCl, pH 7.9, 0.01 M MgCl, 0.15 M KCl, 1 mM DTT and 0.1 mM EDTA, and centrifuged at 200,000g for 12 h. Aliquots (40 µl) of pooled bottom two-third fractions were assayed as in Fig. 1, with the addition of 3.4 µg T4 DNA or 4.0 µg of assayed as in Fig. 1, with the addition of 3.4 µg T4 DNA or 4.0 µg of

poly(dAT).

\*In the activity ratio calculation, it was assumed that UMP appears twice as frequently in poly(dAT)-directed RNA as in T4 DNAdirected RNA. Therefore, the ratio is (pmol UMP in T4 RNA/mg protein × 2): (pmol UMP in poly AU/mg protein). †100% values were: 8,700 c.p.m. for T4 DNA, and 13, 700 c.p.m. for

poly(dAT). ‡Highly purified enzyme from uninfected cells usually showed a ratio

candidate is the 'host shut-off' gene (gene 0.7) as has been suggested3-5. Gene 0.7 transcribes mRNA of molecular weight 650,000 (refs 3 and 10), large enough to code for a protein of the size of I (molecular weight 66,000). The protein product of gene 0.7 is however known to have a molecular weight of only 40,000 although a precursor molecule of larger size may exist3. It is interesting that the product of gene 0.7 was recently shown to be a protein kinase which phosphorylates many host proteins<sup>11</sup>. The possibility that I is a host protein cannot be ruled out. Indeed a protein similar to I in electrophoretic mobility was detectable, although in small amounts, in the RNA polymerase preparations from uninfected cells. It is also possible that gene 0.7 exerts 'host shut-off' function through the protein phosphorylating activity thereby directly or indirectly mobilising I protein.

With the closely related phage T3, a protein which inhibits the host RNA polymerase has been reported12,13 Although this T3 protein seems to be controlled by a late gene, we cannot make a good comparison with I protein because the T3 protein has not been purified.

We have found in addition that I protein also inhibits in vitro translation of MS2 RNA. Figure 3 shows that I protein inhibited MS2 RNA-directed met-tRNA binding to ribosomes (Fig. 3a) and phenylalanine incorporation into protein (Fig. 3b). Using 1.5 µg I protein and 5 µg MS2 RNA, about 50% inhibition was achieved in both cases. Increasing amounts of MS2 RNA reduced the inhibitory effect of I protein; 10 μg of MS2 RNA required about 3 μg of I protein to be inhibited to the same extent. On the other hand, I protein did not inhibit in vitro translation (measured as above) of T7 early mRNA prepared from ultraviolet-irradiated, T7 gene 1-amber mutant phageinfected cells following the procedure described previously\*. These characteristics are very similar to those of a cistron-

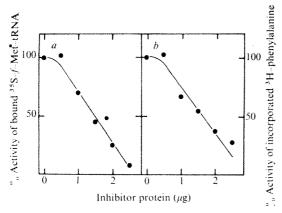


Fig. 3 Inhibition of in vitro translation of MS2 RNA by I protein. RNA-directed f-met-tRNA binding to ribosomes. The initiation factor-dependent reaction was carried out as described previously<sup>8</sup> and contained 5 µg MS2 RNA, 75 µg NH<sub>4</sub>Cl-washed ribosomes, 20 µg crude initiation factors and 3.5 × 10<sup>5</sup> c.p.m. <sup>35</sup>S-labelled *f*-met-tRNA. Various amounts of purified I protein were added as indicated. The reaction was at 35° C for 15 min. The 100% value was 52,159 c.p.m. per reaction. b, MS2 RNA-directed phenylalanine incorporation into protein. A standard in vitro protein-synthesising system<sup>6</sup>. It was confirmed that the major products of this reaction were MS2 coat protein and RNA replicase, by gel electrophoresis<sup>20</sup>. The reaction was at 37° C for 15 min and contained 5 µg MS2 RNA, 10 µCi per 2 nmol <sup>3</sup>H-phenylalanine and various amounts of purified 1 protein. The 100% value was 37,517 c.p.m. per reaction.

specific translational control protein, 'i' factor, which is also one of the host-coded subunits of Q<sub>B</sub> phage RNA replicase (refs 14-18) and is probably identical with the E. coli 30S ribosomal protein, S1 (cited in ref. 16). The 'i' factor binds to the RNA of RNA phages MS2 (refs 14 and 15) and R17 (refs 16-18), and specifically inhibits translational initiation of the coat protein cistron, but it does not inhibit in vitro translation of T4 mRNA or endogenous E. coli mRNA. But 'i' factor seems to have a slightly larger molecular weight (74,000) than the I protein described here. Further investigation is in progress concerning the translational inhibition by I protein and its identity.

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### Biological decay of the 5'-triphosphate termini of the RNA of E. coli

EXPERIMENTS in vitro and in vivo have shown that nascent RNA chains have 5'-triphosphate termini and that the 5' terminal nucleosides are either adenosine or guanosine1-3. Little is known about the fate of these termini. It is known that in Escherichia coli mRNA is degraded rapidly4, and that the nascent precursors of ribosomal and tRNA mature—a process involving trimming at the 5' end<sup>3-7</sup>—and then have only one phosphate group on the 5' end7,8. It seems, therefore, that the 5'-triphosphate termini should disappear as a result of mRNA decay and stable RNA maturation. We have examined the disappearance of triphosphate termini from E. coli RNA and found that they decay at a rate approximately one-half of that of the bulk of the unstable RNA.

We followed the decay of 32P-labelled triphosphate termini in cell cultures after rifamycin SV had been added to stop further initiation of RNA synthesis9. Since E. coli is relatively resistant to rifamycin, we used the mutant strain E. coli H-101 (ref. 10), which is sensitive. To determine whether there was a lag in rifamycin action and how completely it blocked RNA synthesis, a control experiment on induction of the lactose operon was performed. This was similar to that done with strain AS 19 (ref. 11), except that adenosine-3',5'-cyclic monophosphate was added to avoid the effects of catabolite repression<sup>12</sup>. Cells were induced with isopropyl-β-D-thiogalactopyranoside at various times before and after addition of rifamycin, protein synthesis was allowed to continue for 20 min, and

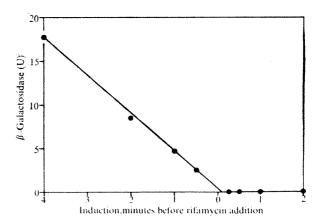


Fig. 1 Inhibition of induction of  $\beta$ -galactosidase synthesis was added to each of 10 flasks containing 1 ml of 0.015 M adenosine-3',5'-cyclic monophosphate (neutralised to pH 7.0) and 0.2 ml of 6 x growth medium. Cells were induced by addition of 0.3 ml of 0.01 M IPTG at various times before or after the addition of 0.6 ml of 500  $\mu$ g ml<sup>-1</sup> of rifamycin SV. Twenty minutes after induction, cells were treated with toluene, and  $\beta$ -galactosidase was assayed by the method of Abbo and Pardee<sup>20</sup>.

 $\beta$ -galactosidase activity was assayed (Fig. 1). The results of this control show that initiation of RNA synthesis was shut off within 15 s of the addition of 100  $\mu$ g ml<sup>-1</sup> of rifamycin.

Decay experiments were performed as follows. Cells, growing at 37°C, were labelled with 32P-phosphate for 5 min, and rifamycin SV was added to stop further initiation of RNA synthesis. Culture samples were taken immediately after rifamycin addition and during the next 16 min. The samples were each mixed with a given volume of cells from a culture labelled with 3H-guanine, to give an internal standard on RNA recovery in the purification steps. RNA was then isolated from the culture samples (see legend to Fig. 2) and purified on gel filtration columns. (This purification was necessary due to the accumulation of 32P-labelled contaminants, presumed to be polyphosphates, which interfere with the detection of the pppNps.) The purified RNA samples were hydrolysed with KOH, releasing adenosine-2'(3')-monophosphate, 5'-triphosphate (pppAp), and guanosine-2'(3')-monophosphate, 5'-triphosphate (pppGp) from the triphosphate termini<sup>13</sup>. There compounds were identified by DEAE paper electrophoresis at pH 3.9 as already described13. 32P was counted in a liquid scintillation counter and corrected to a reference date for radioactive decay.

We performed two experiments on the decay of triphosphate termini, differing in the purification of the RNA by gel filtration. In the first experiment, RNA was purified on a Bio-Gel P-10 column (exclusion approximately 20,000 daltons), retaining the excluded vol-

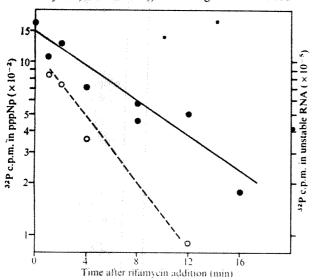


Fig. 2 Radioactivity in RNA (○) and in pppNps (●) after rifamycin treatment. Cells were grown at 37° C in glucose minimal medium³, using HEPES buffer and supplemented with 100 μg ml⁻¹ of methionine. They were grown to an optical density (at 540 nm, 1 cm path) of 0.8, washed with low phosphate medium, shifted to low phosphate medium (2×10⁻³ M phosphate), incubated for 3 min, and pulse labelled with 50 mCi of ³²P-phosphate for 5 min. At the end of this labelling time, rifamycin SV and non-radioactive phosphate buffer (pH 6.8) were added to the culture to make final concentrations of 300 μg ml⁻¹ rifamycin and 10⁻³ M phosphate. Culture samples were taken at various times and mixed with equal amounts of ³H-guanine labelled cells. Cells were broken open in 2% sodium dodecyl sulphate at 60° C and RNA was isolated by phenol extraction, ethanol precipitation and purification on Bio-Gel P-10 (Biorad) gel filtration columns at 4° C. The excluded RNA fractions were then hydrolysed with KOH and the residual DNA and protein were precipitated with perchloric acid¹³. Samples were absorbed on charcoal and eluted with ethanol: 28% NH.OH: H-O (66:2:32). The total ³²P counts in purified ribonucleotides were taken as total RNA and the ³²P counts in pppNps as total triphosphate termini (adjusted by ³H-guanine nucleotide recovery). The data for the decay of RNA were corrected by the subtraction of the stable RNA fraction (that remaining after 16 min).

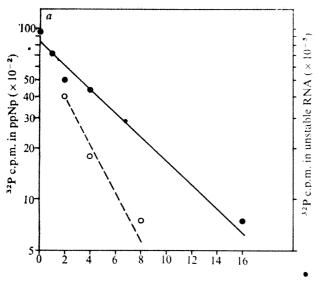
ume for analysis. The data show total 32P c.p.m. in the RNA and in the triphosphate termini as mononucleotides and pppNps released by KOH hydrolysis of that RNA (Fig. 2). The second experiment was similar, except that RNA samples were divided in half, and each half was run on a separate Sephadex G-100 column. eluates from these columns were separately pooled to include both the excluded peak and the material eluting up to a K<sub>av</sub> (ref. 14) of 0.48. Calibration of similar columns, using single-stranded DNA fragments of known length, by Dr Donald A. Kaplan, indicates that this material includes oligomers of length approximately 12 and greater. The data for the second experiment shows 32P c.p.m. in RNA, in total pppNps released by KOH hydrolysis, and also in pppAp with results from duplicate samples given to indicate the variability encountered in these experiments (Fig. 3). Further data, not given, show that the ratio of 32P c.p.m. in pppGp to pppAp at the beginning of each experiment was 1.4 and that this did not change significantly throughout the experiments. Thus the kinetics for the decay of both guanosine and adenosine triphosphate termini were similar.

The data for the decay of RNA and triphosphate termini from these experiments can be approximated by exponential decay as seen by the straight lines drawn on the semi-logarithmic graphs. These approximations give halflives of 3.4 and 6.0 min for the decay of the RNA and triphosphate termini respectively in the first experiment, and 2.1 and 4.6 min in the second experiment. Using a different method, Konrad estimated the halflife of the termini at 21.5° to be at least 170 s (ref. 15).

In interpreting these data, we assume either that the 5' ends of all species of nascent RNA are destroyed by the same mechanism or that the data reflect primarily the decay of a particular species of RNA, presumably the messenger. Preliminary work done in this laboratory by Charles Bieger, indicates that a significant proportion of the 32P-labelled triphosphate termini on E. coli RNA after a 5 min pulse with 32P-phosphate is found in polysomes; however, the exact amount depends on the method of extraction of the cells. If tRNAs were made monocistronically, one would expect their precursors to contribute the majority of triphosphate termini in the E. coli cell because these termini are a measure of the number and not the weight of RNA molecules13. There is, however, an indication that some tRNAs are made polycistronically\*, and thus their contribution to the 5' termini will be reduced. Furthermore, as the nascent tRNA precursors are undetectable in normal cells growing at 37° C, their termini may disappear extremely rapidly. Finally, the dreav of triphosphate termini seen in these experiments could be the result of one of two processes. The 5' end of the RNA could be degraded so that the triphosphate termini would be on a small polynucleotide, for example, less than 12 nucleotides long in our second experiment, or by a process whereby one or more of the phosphates are removed from the triphosphate termini.

Our data rule out the possibility that the triphosophate termini decay much faster than does unstable RNA. In fact the decay of the former could be a result of the decay of the latter. Since the data indicate a longer half life for the decay of triphosphate termini than for that of unstable RNA, they suggest that degradation of triphosphate termini occurs by a separate mechanism than that primarily responsible for the degradation of RNA.

From the results of work on the decay of both lactose mRNA and tryptophan mRNA, Kennell et al. concluded that each cistron in these polycistronic RNAs has a unique site that is vulnerable to attack, causing inactivation. This attack is presumably the initial event in the breakdown of the message<sup>4,18</sup>. From work on the decay of tryptophan mRNA, Yanofsky et al. make a model for the



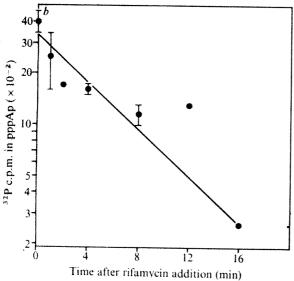


Fig. 3 Radioactivity in RNA (()) and in pppNps (()) after rifamycin treatment. The experiment was performed as described for Fig. 2, except that the RNA was purified on Sephadex G-100 (Pharmacia) columns as described in the text. a, <sup>32</sup>P c.p.m. in RNA and in total pppNp; b, <sup>32</sup>P c.p.m. in pppAp. Upper and lower brackets show data from duplicate columns.

decay of mRNA in which the initial degradative event occurs near the 5' end of the molecule and is followed by degradation in the 3' direction17. We favour a model where internal degradation is the first step in mRNA decay. Such a model is supported by the discovery of 5'-terminal fragments of the transcripts of the T7 early-genes and tryptophan operon<sup>18,19</sup>. The longer half life of the 5' terminus could be the result of the presence of the triphosphate termini inhibiting a 5'-exonuclease attack. Alternatively, if messenger degradation is associated with translation, the 5' end of the molecule, which is not translated, would be degraded by a separate mechanism.

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### Model for wandering restriction enzymes

I wish to propose a model for those DNA restriction enzymes which, although requiring specific recognition sites, make a limited number of cuts at apparently random sites, far from the recognition sites.

Many bacteria have restriction and modification enzymes which recognise and attack DNA from other organisms (for recent reviews see refs 1, 2 and 3). A modification enzyme recognises a particular nucleotide sequence and modifies it, for example, by methylating a nucleotide in this sequence. DNA from other organisms, lacking this modification, is recognised as foreign, and is cut by a restriction enzyme. On the other hand, DNA which has been modified or which does not include the recognition sequence is not cut, that is, it is not restricted. Both strands of DNA are usually modified. Replication of modified DNA yields DNA of which one strand (the parental) is modified, and the other (the daughter) is unmodified. Modification in only one strand prevents restriction but permits modification of the other strand. In this way DNA can replicate without becoming sensitive to restriction.

Restriction enzymes fit into two groups on the basis of their restriction products. Enzymes of group I, such as those of Haemophilus4 and those encoded by the RI5 and RII6 resistance transfer factors, cut at unique sites of the DNA yielding a population of specific fragments7-9 with unique terminal sequences. The terminal sequences, where known, imply that the nucleases act at symmetric DNA nucleotide sequences<sup>4-6</sup>, that is sequences such as

5' . . . pGpTpTpApApCp . . . 3' 3'...pCpApApTpTpGp...5'

which reads the same from left to right as from right to left. This sequence, a substrate of a *Haemophilus* enzyme<sup>4</sup>, consists popipi ) arranged symmetricof two identical components (pGpTpT

Within a symmetric sequence both strands look alike. A modification or restriction enzyme which recognises part or all of the sequence could act on both strands, either sequentially, first on one strand and then on the other, or, if the enzyme were a symmetric dimer, simultaneously on both strands.

Restriction enzymes of group II, those of Esherichia coli<sup>10-12</sup> and phage P1 (unpublished results of K. Horiuchi, quoted in ref. 13), recognise specific sites, but in contrast to group I enzymes they make a limited number of double strand cuts at

apparently random sites, far from the recognition sites. For example, the circular DNA molecule that is the replicative form of phage f1 is cut only once by the *E. coli* B restriction enzyme, yielding a linear molecule which is resistant to further degradation. The cuts generate a heterogenous population of molecules which can be denatured and renatured to reform circular molecules. These circular molecules, in contrast to the linear molecules, are again sensitive to the restriction nuclease, indicating (1) that the recognition sites are not inactivated by the first cut, and (2) that a circular molecule is necessary for the recognition sites to be effective<sup>11</sup>.

A puzzling feature is the difference between the number of cuts introduced and the number of recognition sites. Phage fl has two widely separated recognition sites (denoted sB<sub>1</sub> and sB<sub>2</sub>) which render the phage DNA sensitive to the *E. coli* B restriction nuclease<sup>14</sup>. In vivo, phage bearing mutations in either sB<sub>1</sub> or sB<sub>2</sub> are partially resistant to restriction, and phage carrying both mutations are completely resistant. Although the presence of either recognition site permits a cut by the restriction nuclease, the two sites together do not permit two cuts<sup>11</sup>.

Figure 1 depicts a model for the action of group II restriction nucleases. Suppose that a specific asymmetric (unmodified) recognition sequence permits binding of the nuclease<sup>12,15</sup>. Having bound, the enzyme now wanders (in the direction defined by the orientation of the recognition site) along the DNA until it meets a nuclease which started from an oppositely oriented sequence. A meeting, head to head, is the signal to cut the DNA. The cutting might occur at many different sites, depending on where the nucleases meet. Once the first double strand cut is introduced, enzymes starting from these recognition sites would no longer be able to meet, accounting for the resistance of the linear molecule to further cutting.

In this model the complete recognition site might be viewed as comprising two identical nucleotide sequences arranged in opposite orientations. The stretch of DNA between the two components of the recognition site might be thousands of

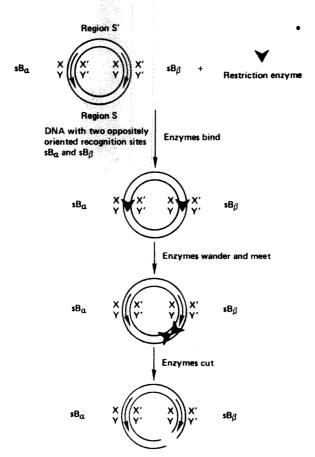


Fig. 1 Model proposed for group II restriction enzymes.

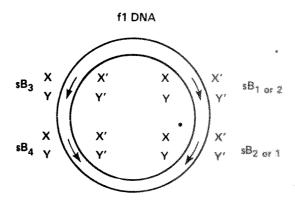


Fig. 2 Proposed arrangement on phage f1 DNA of the recognition sites of the restriction modification system of E. coli B.

nucleotides (the case of phage f!)<sup>11</sup> or only a few nucleotides in which case the restriction enzyme would seem to act like a group I enzyme.

The complete recognition site, composed of two oppositely oriented identical sequences is symmetric. Whereas for group I enzymes, the two components of the symmetric recognition region are juxtaposed, for group II enzymes the two components can be separated by a long and variable nucleotide sequence, and the nuclease can act anywhere in this nonspecific sequence.

Modification (methylation) of the DNA of phage fd (perhaps identical to phage f1) is blocked by mutations at sites sB1 and sB<sub>0</sub><sup>16</sup>, suggesting according to this model, that modification of the putative sites sB<sub>3</sub> and sB<sub>4</sub> depends on the sites sB<sub>1</sub> and sB<sub>2</sub>. The modification enzyme might bind at the recognition site of one strand and wander to a recognition site in the other strand, which it would then modify, thus blocking the binding of restriction enzyme and protecting the DNA. Two oppositely oriented recognition sites would be required for modification activity, as for restriction activity. The type of interaction between the modification enzyme and recognition site would depend on how each component is presented. Free enzyme would bind to but not modify a recognition site. Wandering enzyme would modify a recognition site presented in the orientation opposite the orientation of the site to which that modification enzyme had bound.

The restriction nuclease might also interact differently with the two components of the recognition site. According to the version presented in Fig. 1, the restriction nuclease might bind at one recognition site and wander past the complementary site, finally encountering the complementary nuclease in the region S'. Cutting in the region S' should not preclude cutting in the region S. Only one cut is observed, however, for f1 DNA, suggesting that the restriction nuclease cannot pass a recognition site, perhaps because of the particular experimental conditions used, or perhaps because wandering restriction nuclease specifically interacts with a recognition site to obviate further wandering.

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### C-type viruses in systemic lupus erythematosus

VIRUSES have been implicated as the agents of systemic Jupus erythematosus (SLE) expressed by dogs' and mice<sup>2</sup>. We have shown that cell-free filtrates prepared from the spleens of dogs with positive LE cell tests induced antinuclear and anti-double-stranded DNA antibodies in CAF mice. The canine filtrate also induced lymphoid tumours in some recipient mice<sup>1</sup>. One of these neoplasms, SP 104, produces a monoclonal antibody against double-stranded DNA. Cultured SP 104 cells produce, in addition to the anti-DNA antibody, C-type RNA viruses that induce antinuclear antibodies when inoculated into normal mice (R.M.L., W.T., C.S., and R.S.S., unpublished). Here we present preliminary evidence linking the SP 104 virus to SLE in humans. Our results suggest that antigenic determinants related to the SP 104 virus are present on the lymphocytes of patients with SLE.

An antibody to the SP 104 virus was made in rabbits using purified C particles that were isolated from the fluid of cultured SP 104 cells by isopycnic centrifugation3 through a 15-60% sucrose gradient. The virus had a buoyant density of 1.16 and all the reverse transcriptase activity of the preparation was localised to this band of the gradient. The banded virus contained 70S RNA, behaved as a B-tropic MuLV in the XC plaque assay, contained the gs-1 antigen of MuLV, had a PI of 3.2 by isoelectric focusing and by electron microscopy consisted almost entirely of C-type particles.

The purified virus was disrupted with ether and injected into New Zealand rabbits. Ten days after two subcutaneous injections of the virions, serum was collected and absorbed sequentially with foetal calf serum, normal murine (CAF<sub>1</sub>) spleen cells and blood lymphocytes from normal human subjects. By gel diffusion analysis, a single line of identity was obtained when purified undisrupted and ethertreated SP 104 virions were reacted with the absorbed serum. The absorbed serum failed to react with DNA in gel diffusion tests and did not bind to 3H-labelled DNA in a sensitive radioimmunoassay for anti-DNA antibodies3. Neither antinuclear antibodies nor antibodies against human or murine serum proteins or cell membrane were detectable in the rabbit serum.

A globulin-rich fraction of the absorbed serum was prepared by ammonium sulphate precipitation. The final product reacted with SP 104 virions in gel diffusion and failed to react in any of the above mentioned tests. The globulins were then conjugated to fluorescein isothiocyanate in a molar ratio (fluorescein: protein) of 10:1 (ref. 7). Lymphocytes, separated from the blood of subjects by centrifugation through a Ficoll-Hypaque gradient<sup>6</sup> and further purified by removal of phagocytes8, were incubated with the conjugate, washed, fixed in ethanol and examined by dark field fluorescence microscopy. The lymphocytes of normal subjects failed to react with the conjugate. By contrast, typical and brilliant membrane fluorescence was observed on the lymphocytes of five out of six patients with SLE. The number of lymphocytes stained by the conjugate ranged from 46×10<sup>s</sup> to 400×10<sup>s</sup> cells. Individual values for the patients studied were 0, 46, 149, 181, 195, and 400. These figures were obtained by examining at least 200 cover slip fields, each of which contained between 400 and 800 lymphocytes. Independent experiments performed on the same cell preparations by two of us gave results that agreed within a margin of 20%. Normal rabbit globulins, treated in the same way, failed to react with any of the lymphocytes.

We next sought evidence in a patient with SLE of an antibody with reactivity similar to that of the rabbit anti-SP 104 virus antibody. A 12-yr-old girl (DeV) was selected as a subject because she had active, untreated SLE with multisystem involvement. Her serum was treated with ammonium sulphate, conjugated to fluorescein isothiocyanate, and used to stain the blood lymphocytes of various subjects in the same manner as the rabbit serum. DeV's serum failed to bind to the cytoplasmic membranes of lymphocytes from either six normal subjects or from six patients with diseases other than SLE. By contrast, typical membrane staining was found with the lymphocytes of eight out of ten patients with SLE (Table 1). The number of lymphocytes stained by the conjugate ranged from 6×10° to 650×10° lymphocytes, a proportion similar to that observed with the rabbit antiserum. Raising the temperature of the reaction mixture to 37° C for 30 min (following incubation at 4°C) resulted in polar capping of the fluorescent stain. The reactivity of the DeV reagent was abolished by previous incubation with a monospecific rabbit anti-human IgG serum, but not by incubation with a rabbit anti-human fibrinogen serum. Immunoglobulins prepared in the same way from normal human serum did not bind to any of the lymphocytes of five patients with SLE.

The immunoglobulin fraction of DeV's serum was absorbed with pelleted C-type particles obtained from the SP 104 culture. The absorbed material, after conjugation to fluorescein isothiocyanate, failed to stain the lymphocytes of five out of eight patients with SLE. In a seventh patient (T.O.U.), absorption of DeV's serum with C-type particles reduced the reaction by about 90%. Absorption of the DeV conjugate with a suspension of 600×106 normal murine spleen cells failed to diminish its reaction with lymphocytes of patients with SLE. Moreover, the DeV conjugate failed to stain the cytoplasmic membranes of normal murine spleen cells. Removal of all anti-DNA antibodies by absorption of the conjugate with double- and single-stranded DNA also failed to diminish its ability to bind SLE lymphocytes, except in two cases, which are discussed presently. In a preliminary experiment, we also found that absorption with yeast (single-tranded) RNA and poly(I)-poly(C) (doublestranded RNA) also failed to remove the activity of DeV's serum against SLE lymphocytes.

Three of the eight patients we tested (L.E.V., F.A.Z., and T.O.U.) differ from the others in that they were clinically ill when their lymphocytes were examined; that relatively large numbers of their lymphocytes reacted with the DeV conjugate, the anti-SP 104 virus serum, or both; that absorption of this reactivity by the SP 104 virus was incomplete and that absorption by DNA removed some of the activity (F.A.Z., and T.O.U.). These results indicate that more than one SLE-related antigen may

		Absorption with:						
SLE Patient	DeV conjugate	SP 104	D-DNA	S-DNA				
C.R.O.	0		- Secretario	and the same				
H.E.A.	0	and the same of	opaperson sine					
B.O.U.	6	0	44	30				
H.I.E.	20	0	13	30				
H.U.N.	92	0	166					
C.O.O.	61	0	NAME OF TAXABLE PARTY.					
R.U.G.	36	0	32	-2020004				
L.E.V.	356	187	275	173				
F.A.Z.	350	202	256	62				
T.O.U.	650	44	410	76				

SP 104, the pellet containing C-type virus particles; D-DNA, double-stranded DNA; S-DNA, single-stranded DNA.

appear on the membranes of circulating lymphocytes during the acute phase of the illness.

The antibody we detected in DeV's serum is not the anti-lymphocyte antibody described in SLE (ref. 9), nor is it an anti-IgG that stains B cells10 since it does not react with normal lymphocytes. We have tried to stain PHAtransformed lymphocytes from normal subjects with the DeV reagent, but no reaction was detected. The activity is not due to an antibody against the Epstein-Barr virus as no anti-EBV activity in DeV's serum has been found. Moreover, the determinant on the test lymphocyte is not an anti-IgG that might bind to the DeV reagent's because conjugated normal IgG did not react with the cells. We do not know if DeV's serum is unique; tests with the sera of other patients with SLE are in progress.

It seems that the lymphocytes of patients with SLE express an antigenic determinant shared by C-type RNA viruses produced by the SP 104 tumour. This plasmacytoma, we stress, also produces an antibody against double-stranded DNA; such antibodies are characteristic of SLE. Experiments are now under way to determine if the SP 104 virus is unique in its relationship to SLE. Our results do not exclude a virus-induced cellular antigen. Results with the rabbit antibody, however, which is directed against purified virus, suggest the presence of a virion determinant on the cytoplasmic membranes of lymphocytes of patients with SLE

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### Rapid in vivo assay for murine lymphatic leukaemia viruses

SEVERAL rapid in vitro methods1-5 for the quantitative assay of murine lymphatic leukaemia viruses (MuLV) have replaced the once standard in vivo bioassay for leukaemogenicity8,7, which was slow, cumbersome, and relatively costly. The in vitro procedures, however, suffer from two limitations: first, some MuLV propagated serially in vitro may lose leukaemogenicity while retaining infectivity in vitro\*, so the in vitro assays do not necessarily correlate with biological activity invivo; second, some wild-type MuLV extracted from normal or neoplastic tissues in vivo seem to require a period of adaptation to in vitro growth conditions before they can be assayed reliably by in vitro methods9.

Both of these limitations apply to the radiation leukaemia virus (RadLV), a naturally occurring MuLV consistently extractable from thymic lymphomas induced in strain C57BL mice by whole-body X-irradiation, which induces thymic lymphomas after inoculation into neonatal or infant C57BL/Ka mice (in vivo-derived wild-type RadLV)6,16. It can also be propagated on mouse embryo fibroblasts8 and collected from the supernatant of permanently infected cultures (in vitroderived RadLV, designated RadLV\*). Several of the rapid • in vitro assay methods have recently been adapted to the titration of RadLV\* (refs 9, 11 and 12 and A.D., O.N., E. Gerlmann, and H.S.K., unpublished). Here we report a rapid in vivo endpoint dilution assay for wild-type RadLV which compares in sensitivity with in vitro endpoint dilution methods, correlates well with leukaemogenicity bioassays, and should be equally applicable to the in vivo assay of other thymotropic MuLV in their susceptible host strains.

C57BL/Ka mice of both sexes were used at 4-5 weeks of age. Wild-type preparations of RadLV were extracted from RadLVinduced lymphoma tissue by a previously described procedure18. Serial five-fold dilutions of virus in medium containing Polybrene (Abbott Laboratories), 4 µg ml<sup>-1</sup>, were injected in a volume of about 0.02 ml per lobe into one or both thymic lobes of groups of test mice14. A group of mice was also injected intravenously with 0.2 ml of serially diluted virus. Thymus cell suspensions were prepared at serial time intervals thereafter. Mice were killed with ether. The thymus was dissected free, minced in a small Petri dish, and passed through nylon cloth into (0.175 M NaCl) saline. Cells were kept in suspension on ice, and their concentration was adjusted to  $1 \times 10^6$  cells ml $^{-1}$ .

Indirect immunofluorescence (IF) staining of acetone-fixed cells for the detection of MuLV antigens was carried out as described by Hilgers et al.4 and adapted to virus titration

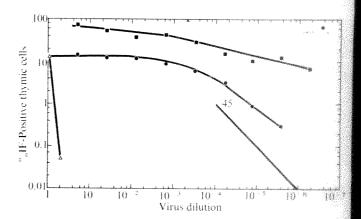


Fig. 1 Titration curves for serial dilutions of RadLV injected intrathymically into one ( ) or both ( ) lobes of the thymus, or injected intravenously (A). A 45° slope curve is included for comparison.

Time after					Virus di	ilutions				
inoculation	59	5-1	5-2	5 -3	5 -4	55	5 -6	5 -7	5-8	5 -1
1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	NT	NT
4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	NT	NT
7	15,16	11,12, 14,15	10,12, 13,15	4,7,10,14	1,7,8,9	2,3,3,4	0.7.1.0, 1.0,1.0	0.0, 0.0, 0.5,0.7	NT	NT
14	66,69	74,82, 84,90	50,74 86,92	73,74, 90,96	60,65, 90,92	25,51 54,69	23,29, 45,64	0.0,7.5, 9.0,63	0,0,0,60	0,0
21	70,72	72,80, 85,90	79,83	70,77	70,80	69,78	65,72	0,78,87	0,0,0,0	0,0,0,
28 Total no.	NT*	NT	NT	NT	NT	NT	NT	60,80	0,90,90	0,0,0,
nice tested No. positive er 14 or more	10	16	14	14	14	14	14	17	11	10
days	4/4	8/8	6/6	6/6	6/6	6/6	6/6	7/9	3/11	0/10

<sup>\*</sup> Not tested.

in vitro by Declève et al.9. The percentage of IF-positive cells recorded in each assay was that observed at the highest concentration of rat anti-serum yielding fluorescence specific for virus-related antigens<sup>4,9</sup>. Counts of 100-200 total cells suffice when IF-positive cells are abundant; conversely, fields containing 20,000 or more cells may be scanned to detect low frequencies of IF-positive cells. Direct examination of thymic lymphoid tissue for viral antigens by immunofluorescence was found to be straightforward, since no nonspecific reaction with immunoglobulin-bearing lymphoid cells could be detected in the normal rat serum or saline controls.

After direct intrathymic (IT) injection of RadLV into one thymic lobe, virus-related antigens were first detected at 7 d for RadLV dilutions ranging from  $5^{\circ}$  to  $5^{-7}$  (Table 1). At 14 d after inoculation, 3 of 11 mice were positive at the  $5^{-8}$  dilution, and 0 of 10 at the  $5^{-9}$  dilution, yielding an endpoint titre of  $3.9 \times 10^{5}$  per 0.02 ml. When the percentage of IF-positive cells observed on day 7 after inoculation is plotted against virus dilution on a log-log scale (Fig. 1), a plateau value of 12-15% for dilutions  $5^{\circ}-5^{-3}$  is seen, followed by a decrease in percentage IF with increasing virus dilution. The slope of the curve is less than  $45^{\circ}$ .

When both lobes of the thymus were injected with 0.02 ml each of RadLV, virus-related antigens were detected as early as 2 d after inoculation of high concentrations of virus. The percentage of positive cells in all positive mice at one week was above 2%; among the negative mice, no positive cells were found among more than 20,000 examined. The endpoint titre for the  $5^{-9}$  virus dilution was detected by 7 d (endpoint titre  $1.9 \times 10^6$  per 0.04 ml). The curve obtained when the results were plotted on a log-log scale (Fig. 1) again has a slope much less than  $45^\circ$ , thus ruling out the interpretation, based on the results of unilobar injection, that the reduced slope might be due to selective virus-induced involution of the injected lobe 16. Instead, the slope of less than  $45^\circ$  is apparently due to secondary infection; accordingly, the assay yields valid results only as an endpoint dilution procedure.

In previous studies using the leukaemia induction bioassay<sup>6.7</sup>, it was shown that the intravenous (IV) route of viral injection is about 100 times less sensitive than the IT route. The same difference in sensitivity has now been observed with the new IF assay. At 3 week after IV injection of serial RadLV dilutions, the IF endpoint titre was  $3.1 \times 10^3$ , about 100 to 1,000 times less than that obtained after intrathymic injection.

Up to now the bioassay method<sup>6,7</sup> has been the only assay by which one could determine how many leukaemogenic particles were present in a given volume of RadLV preparation. To obtain a direct comparison of the two *in vivo* assays, the same stock of virus used for the unilobar IF assay described above was inoculated IT (0.02 ml) into one thymic lobe of C57BL mice. The endpoint was scored after 9 months

by determining the highest dilution of RadLV which induced lymphomas. The endpoint dilution was  $5^{-8}$  (titre  $3.9 \times 10^5$ ), in excellent agreement with the titre obtained after unilobar injection in the *in vivo* percentage IF assay. Several replicate *in vivo* IF assays, using IT injection, have indicated good reproducibility of the method.

The estimated titres obtained by three *in vitro* assay methods are as follows: the reverse XC cell test<sup>11</sup>, the percentage IF assay<sup>9</sup>, and the UCI-B focus assay<sup>5.13</sup> were comparable  $(1.9 \times 10^6 \text{ per } 0.4 \text{ ml})$  to those obtained with the new *in vivo* percentage IF assay.•The direct XC test<sup>3</sup>, however, and the indirect helper focus assay<sup>12</sup> gave lower  $(1 \times 10^5 \text{ per } 0.4 \text{ ml})$  and higher  $(9.6 \times 10^8 \text{ per } 0.4 \text{ ml})$  titres, respectively. *In vivo*-derived virus preparations have been more difficult to assay by rapid *in vitro* methods because major differences exist in viral growth kinetics between the *in vitro*-derived and *in vivo*-derived preparations of RadLV.

The new *in vivo* assay is direct, rapid, and sensitive. The highest dilution of RadLV capable of inducing a lymphoma is also the highest dilution capable of infection and active virus replication in thymus cells, as revealed by the appearance of virus-related antigens in the cell cytoplasm. The assay should therefore prove useful in elucidating the sequence of events *in vivo* between the time of virus inoculation and the ultimate development of lymphomas induced by this and other thymotropic MuLV.

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### Sensitivity of cytotoxic T cells to T-cell mediated cytotoxicity

LYMPHOID cells sensitised against alloantigens can lyse in vitro target cells bearing these alloantigens1,2. In most of the experimental systems using in vivo sensitised cells, T cells (thymus-dependent lymphocytes) are both necessary3 and sufficient<sup>4,5</sup> for induction of target cell lysis. This seems to apply also to in vitro sensitised cells<sup>6-9</sup>. Specific cytolysis can be described as a two stage process: first, the recognition of target cell antigens by specific receptors at the surface of the 'killing' cell10,11; and, second, lysis itself.

Speculations about the mechanism of the second stage vary between two extreme hypotheses, that is, target cell lysis being exclusively due, either to direct membrane-membrane interaction with the killing cell, or to the effect of a nonspecific cytotoxic factor, a 'lymphotoxin', released in the surrounding fluid by the killing cell. Direct search for this or similar factors provided contradictory results<sup>12-23</sup>. To investigate the possibility of a lymphotoxin involvement, I resorted to a different approach.

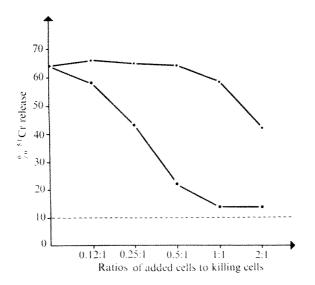


Fig. 1 Cytototoxicity, expressed as <sup>51</sup>Cr release, of mixtures of k anti-d killing cells and added cells. A constant amount of killing cells (2×106) was incubated overnight in the presence of various amounts of added cells, either normal d cells ( $\bullet$ ) or d anti-kcells ( ). The surviving cells were tested for their cytotoxicity against d target cells, as indicated in the legend of Table 1. -, Spontaneous release by target cells alone.

Table 1 Mixtures of k anti-d killing cells and normal or d anti-kadded cells: percentage of surviving cells and residual cytotoxicity for d target cells

Ex	cp. Added	Cytotox Ratio	cicity† for sof ad	ded to	rget ce killing	lls Ratio	s of ad		celis! Killing
	cells*	0:1§	0.5:1 65	1:1 60	2:1 49	0:1	0.5;1	1:1	2:1
1	d anti-k	54	36	9	3	• 80	N	iot dos	ie
,	normal $d$	46	52	48	35	70	43	35	40
2	d anti-k	40	6	1	2	70	30	25	23
	normal $d$		60	52	43	e ir	27	30	47
3	d anti-k	54	14	2	1	55	45	20	27

C3H anti-BALB/c (k anti-d) and BALB/c anti-C3H (d anti-k) sensitised cells were obtained after 5 d of coincubation at 37° C in a 10% CO<sub>2</sub> atmosphere of  $7\times10^6$  responding spleen cells and  $7\times10^6$  irradiated (2,000 rad) stimulating spleen cells in 2.5 ml of modified Factor and 10% had been spleen cells in 2.5 ml of modified Eagle medium plus 10% heat-inactivated foetal calf serum, in the presence of 10<sup>-6</sup> M mercaptoethanol<sup>27,28</sup>, per well of FB-16-24-TC Linbro plates.

\*Added cells were either normal BALB/c (d) spleen cells or Added cells were either normal BALB/c (a) specificells of sensitised d anti-k cells. They were transferred into type 2003 plastic Falcon tubes, in 1 ml of medium plus serum, with  $2 \times 10^5$  k anti-d sensitised killing cells, at the indicated ratios of added cells to killing cells. After one centrifugation at 180g for 5 min, the tubes were incubated overnight at 37°C and centrifuged again. The supernature were principled out 1 ml of fresh medium this serum was added as were pipetted out, 1 ml of fresh medium plus serum was added per tube and the resuspended cells were tested for their cytotoxicity and

counted in a trypan blue exclusion test

†Cytotoxicity was measured as follows. Target cells were P815 mastocytoma cells (d), labelled overnight with 100 μCi of <sup>53</sup>Cr in 1-2 ml as previously described<sup>s</sup>, washed twice and distributed in V-shaped wells of IS-MVC-96 Linbro microplates (10<sup>4</sup> P815 cells per 0.1 ml per well). Each well also received 0.1 ml of one of the cell suspensions tested for their cytotoxicity. The microplates were centrifuged (280g, 2 min) to increase the sensitivity of the cytotoxicity test<sup>5,29</sup>, incubated for 4 h at 37° C in a humidified CO<sub>2</sub> atmosphere and centrifuged again. Volumes of 0.1 ml of supernatants were sampled with an Eppendorf micropipette, their radioactivity was measured and expressed as % radioactivity initially present in 10<sup>4</sup> P815 target cells. Cytotoxicity, in this table, is expressed as % <sup>53</sup>Cr-release averaged from triplicate wells, minus 10% which was spontaneous <sup>51</sup>Cr-release his transfer of the second of t taneous  $^{51}$ Cr-release by target cells alone in each of these three experiments. The cytotoxicity for d target cells of d anti-k added cells alone was, for experiments one to three, 3, 3, and 2, respectively. This shows the degree of specificity of these anti-k cytotoxic cells. In this type of assay, an experimental difference of 5% or more is statistically significant  $(P=0.05)^{11}$ .

Percentage of trypan blue-excluding cells after overnight incubation, compared to the initial number of trypan blue-excluding cells in the mixtures of added cells and killing cells. These figures can be used to determine the experimental parameters not given in the table. For instance, for experiment three, column 0.5:1, the initial number of cells was  $10^6$  added normal cells plus  $2\times10^6$  killing cells, thus  $3 \times 10^6$  cells. The percentage of surviving cells was 27%, the number of surviving cells was thus  $8 \times 10^6$ . One tenth of this was used for the cytotoxicity test, giving a ratio to target cells of 8:1. The corresponding cytotoxicity was 60.

§Killing cells incubated alone, without added cells.

Let us provisionally make the hypothesis of a nonspecific cytotoxic factor, released into the extracellular fluid by the killing cell upon specific contact with the relevant target cell, which would be necessary and sufficient for subsequent target cell lysis. This extracellular cytotoxic factor would lyse the target cell, and should eventually lyse the cytotoxic cell as well. The cytotoxic cell is not lysed, however, either during or after the killing process 24,25, and is able to kill other target cells 2,3,28. The cytotoxic cell must then be resistant to the factor. If the cytotoxic cell (A) is subjected to the effect of other cytotoxic cells (anti-A), the anti-A cells would, by hypothesis, act against A cells through the release of a soluble factor. A cells, being resistant to the factor, must be resistant to the effect of anti-A cells as well. In other words, our initial hypothesis about a soluble factor leads to the prediction that cytotoxic cells should be resistant to other cytotoxic cells. In fact, I found that cytotoxic T cells are sensitive to the effect of other cytotoxic T cells specifically directed against them. This sensitivity, contrasting with the survival of the cytotoxic cell when it kills a target cell, argues strongly against an 'all lymphotoxin' mechanism accounting for the lytic event.

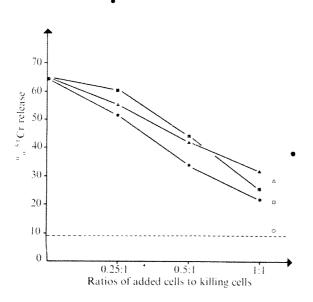


Fig. 2 Cytotoxicity, expressed as  ${}^{51}$ Cr release, of mixtures of  $b \times k$  anti-d killing cells and added cells. A constant amount of killing cells  $(2 \times 10^6)$  was incubated overnight in the presence of various amounts of added cells, either b anti-k ( $\blacksquare$ ), or k anti-b ( $\blacktriangle$ ), or d anti-k ( $\blacksquare$ ). The surviving cells were tested for their cytotoxicity against d target cells.  $\triangle$ ,  $\square$  and  $\bigcirc$  indicate cytotoxicity of added cells alone; ---, spontaneous release by target cells alone.

**Table 2** Mixtures of  $b \times k$  anti-d killing cells and either no or normal or sensitised added cells: percentage of surviving cells and residual cytotoxicity for d target cells

Exp. Added Ratios of k	illing to cells 1 2:1 53	added	Ratios 0.5:1	surviv of kill cel 1:1 70	ing to	added 4:1
cells	cells 1 2:1 4 53	4:1 57	Ratios 0.5:1 80	of kill cel 1:1	ing to ls 2:1	added 4:1
* * * * * * * * * * * * * * * * * * * *	1 2:1 1 53	57	80	1:1	2:1	
	53	57	80			
0.5:1 1:				70	70	70
none* 31 44	3 55	60			/0	78
1 normal b 34 48		20	53	75	73	52
<i>b</i> anti- <i>k</i> 6 10	25	48	40	30	30	44
none 20 33	43	52	40	60	55	55
2 normal b 21 36	46	52	40	35	33	48
<b>b</b> anti-k 5 12	29	45	27	30	37	48
none 41 53	59	60	80	80	50	35
3 normal k 53 60	) 66	64	47	40	43	46
k anti-b 11 36	5 58	65	20	20	30	24
none 25 40	) 45	45	80	60	70	55
4 normal k 39 4:	5 50	50	47	40	40	48
k anti-b 8 26	5 45	47	27	20	33	46

Experimental protocol similar to the one given in legend of Table 1, except that a constant amount of added cells ( $10^6$ ) is incubated overnight with varying amounts of killing cells. Killing cells were (C57BL/ $6 \times CBA$ ) F, hybrid anti-BALB/c cells. Added cells were either normal C57BL/6, or normal AKR, or C57BL/6 anti-C57BL/6 cells. For experiments one to four the spontaneous  $^{51}$ Cr-release by target cells alone was 9, 10, 12 and 8, respectively, and the cytotoxicity above spontaneous release of sensitised added cells alone was 5, 2, 10 and 8, respectively, which shows the degree of cross-reactivity of k anti-b cells in particular towards d target cells.

\*Added cells were not included. In these experimental groups the amounts of killing cells were the same as in the corresponding groups with added cells.

In a first set of experiments, C3H anti-BALB/c (H-2k anti H-2d; k anti-d for short) killing cells were incubated overnight together with either d anti-k or normal d 'added' cells. The residual anti-d cytotoxic activity of the mixtures in three consecutive experiments is given in Table 1. Cytotoxicity is much more depressed when d anti-k cells are added instead of normal d cells. An additional experiment showed (Fig. 1) that eight times more normal cells were necessary to depress cytotoxicity to the same extent as anti-k cells. The difference between both types of added cells was significant even at a ratio as low as 0.12 added cell to one killing cell, corresponding in this experiment to a final ratio of less than one added cell to one target cell. These results would be consistent with the possibility that the k anti-d killing cells are lysed or inactivated by the anti-k added cells, but other types of cell interactions, such as mechanical blocking30, booster or 'interkill' effects, may also be involved.

In a second set of experiments, I therefore used another strain combination. (C57BL/ $6 \times$  CBA)  $F_1$  hybrid anti-BALB/c killing cells ( $b \times k$  anti-d) were incubated overnight together with either d anti-k, or b anti-k, or k anti-b added cells. Use of the latter two types of added cells eliminates the possibility of blocking or interkill. A profound anti-cytotoxic cell effect of added cells was readily obtained in various experimental situations, for example when a constant amount of killing cells was preincubated with various amounts of added cells (Fig. 2), or when a constant amount of added cells was preincubated

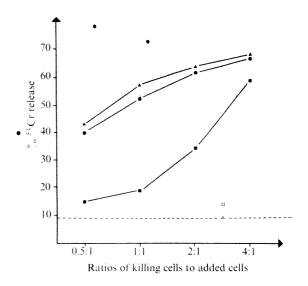


Fig. 3 Cytotoxicity, expressed as  ${}^{51}$ Cr release, of mixtures of  $b \times k$  anti-d killing cells and added cells. Various amounts of killing cells were incubated overnight either alone ( $\bullet$ ) or in the presence of a constant amount of added cells (10 ${}^{6}$ ). Added cells were either normal b cells ( $\bullet$ ) or b anti-k cells ( $\bullet$ ).  $\Box$  and  $\triangle$  indicate cytotoxicity of added cells alone.  $\frown$ , Spontaneous release of target cells alone. More complete data for this experiment are given in Table 2, experiment 1.

with various amounts of killing cells (Fig. 3 and Table 2). Clearly, incubation of  $b \times k$  anti-d killing cells with either k anti-b or b anti-k added cells led to a much lower residual anti-d cytotoxic efficiency than incubation with normal added cells. Finally experiments with anti- $\theta_{AKR}$  antiserum showed that the effect of k anti-b added cells on  $b \times k$  anti-d killing cells could be abolished by pretreatment of the added cells with anti- $\theta$  antiserum plus complement, but not with anti- $\theta$  antiserum or complement alone.

Addition to killing cells of cells specifically sensitised against them leads to a decrease of the cytotoxic efficiency of the killing cells. This is consistent with the possibility that cytotoxic

cells can be lysed by other cytotoxic cells. These cytotoxic cells may not be lysed, however, but merely functionally inactivated. Some degree of cell lysis definitely occurred in mixtures of killing cells and sensitised added cells, in which the number of surviving cells was usually lower than in mixtures of killing cells and normal added cells. In any case, even functional inactivation of killing cells without lysis, and a fortiori lysis itself, mean that killing cells are sensitive to the effect of other cytotoxic T cells directed against them.

This result is contrary to the prediction derived from my initial hypothesis of a lymphotoxin-mediated lysis. Complete rejection of this hypothesis, however, would be founded on the formal line of reasoning given above, which may be endangered by any parameter not included in its premises (for instance, resistance of cytotoxic cells limited to the short time when they actually kill, or existence of a different mechanism for lysis of tumour target cells and lymphoid cytotoxic cells). Still, the present report makes it much less likely that the lytic phase of specific T-cell mediated cytotoxicity is an 'all lymphotoxin' one, that is, one entirely accounted for by a nonspecific extracellular soluble factor. This does not exclude a role for soluble factors, which may still be involved, for instance if directly injected by the killing cell into the target cell, or as an extracellular agent completing membrane-induced lesions. Alternatively, no soluble factors at all may be involved, cytolysis being exclusively due to membrane-membrane interactions. Whatever the lytic mechanism turns out to be, because of killing cell sensitivity it would have to include a stage polarised towards the target cell.

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### Effects of cytotoxic sera on endothelium in vitro

THE rapid rejection of xenografts between widely different species, and the rapid 'hyperacute' rejection of vascularised allografts to previously sensitised recipients are both thought to be due to the effects of cytotoxic antibodies in recipient serum1.2. I have used cultured vascular endothelium to observe the direct effects of cytotoxic sera in vitro. Here I examine the effects on pig endothelium of dog serum (as a model for xenograft rejection) and of pig alloantisera to PLA antigens3 (as a model for hyperacute allograft re-

Endothelium was obtained from the aortae of five pigs of known PLA type by brief surface digestion with collagenase'. Shortly after death the aorta was cannulated by the left subclavian artery and flushed with cold saline. The vessel was dissected out from aontic arch to diaphragm, the intercostal arteries were ligated and a 4 F. G. Portex cannula was tied into each end. It was filled with a warm 0.2 w/v solution of Worthington collagenase (about 500 units ml-1) in tissue culture medium (TCM-Waymouths medium, Burroughs Wellcome, plus 10% heat inactivated foetal calf serum)—and incubated at 37° C for 15-20 min. Detached cells were flushed out with saline, washed with TCM, and plated out in glass 'medical flat' bottles. When required, the cells were resuspended with collagenase and plated at high cell density (150-200×10<sup>3</sup> ml<sup>-1</sup>) on to 13 mm diameter glass coverslips in plastic Bijou bottles containing 1 ml of medium. A confluent monolayer was usually obtained in 24 h (Fig. 1a). The coverslips were transferred to 30 mm plastic Petri dishes or to 'Sterilin' slide culture chambers for observation by inverted phase-contrast microscopy. Pigs were tissue typed on the basis of lymphocytotoxic assays using sera raised by skin grafting, kidney grafting or the injection of lymph node cells3. Baby rabbit serum was used as a complement source, after testing to exclude intrinsic cytotoxicity. Six different alloantisera were used to produce cytotoxic effects on the endothelial cultures. Experiments were done in duplicate; negative controls of pooled normal serum and compatible alloantisera were included. Fresh dog serum was obtained from three adult mongrels. Heat inactivated dog serum (56° C for 30 min) was used as a control. Pig and dog platelets were prepared from citrated blood by differential centrifugation. The platelet button was allowed to stand for 1 h at 37° C. washed in calcium-free TCM, and resuspended in TCM at a concentration of 500×106 ml<sup>-1</sup>.

Pig kidneys transplanted into dogs show a rapid decline in blood flow within a few minutes of vascularisation. The histological appearances are of almost total obliteration of the smaller vessels by thrombi containing platelets, fibrin and leukocytes. The time course can be slowed by perfusing the kidney with diluted or platelet depleted blood,

or with blood previously passed through a pig liver. Pig endothelium incubated with fresh dog serum showed changes within 15 min in each of four experiments with different endothelial lines, and the monolayer was completely disrupted within 90 min. The first change is that the endothelial cells become more rounded, and draw away from each other so that the cement lines after silver staining, which indicate close intercellular apposition, can no longer be demonstrated. The cells appear granular and vacuolated; many of them extrude cytoplasm as 'ghost bubbles'. At 90 min most of the cells have detached from the substrate and floated away. (Fig. 1b).

The damaged cells become 'sticky' to platelets-resuspended cells treated with dog serum and then mixed with

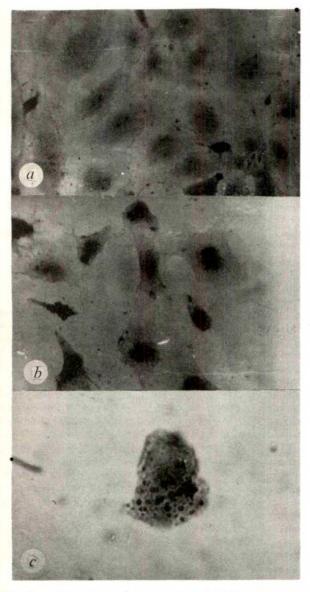


Fig. 1 a, Monolayer of normal pig endothelium in culture, stained silver and haematoxylin; b, pig endothelium treated for 30 min with 'lymphocytotoxic' antiserum, then for 35 min with baby rabbit serum; c, pig endothelial cell surrounded by platelets. About 75,000 resuspended endothelial cells were spun down and treated with 50  $\mu$ l of specific pig anti-PLA serum for 30 min, washed, treated with 100 µl rabbit serum for 30 min and added to 0.5 ml of pig platelet suspension. The mixture was gently shaken for 5 min in a siliconed tube. Smears were made and stained with Giemsa.

a dog platelet suspension become surrounded by platelets (Fig. 1c). Dog serum contains a heterophil antibody which agglutinates pig red cells and will cause their lysis in the presence of complement'. Adsorption of dog serum with pig red cells at 4° C renders it innocuous to pig endothelium, and pig endothelial cells incubated with heat inactivated dog serum will form cross agglutination rosettes with pig red cells.

Exactly comparable effects can be produced by incubating pig endothelium with 'lymphocytotoxic'-alloantisera and then treating it with rabbit serum as a complement source (pig serum is also effective, but some samples are 'anticomplementary'). In five experiments with different endothelial lines, changes were observed after 30 min and were marked at 60 min. Sera toxic to lymphocytes from a given pig were in each case toxic to endothelium from the same pig, but antisera to other specificities, pooled normal serum and specific antibody in the absence of complement were not. The damaged cells were again 'sticky' to pig platelets (these were obtained from pigs compatible with the antiserum used, to avoid the possibility of cross agglutination). The platelet-endothelial cell aggregates are similar to those described by Rafelson et al. after treatment of platelet-endothelial cell mixtures with thrombin6, and may be related to the platelet thrombi observed in vivo.

Similar effects on human endothelium have been observed as a result of treatment with antibodies to HL-A antigens (D. de B. and V. Joysey, in preparation).

I thank Professor R. Y. Calne, Mr M. Slapak, Dr V. Joysey and Mr D. White for help and advice; Mr D. White for providing tissue-typed pigs and pig antisera, and Mrs S. Rogers for providing dog blood. I am grateful to the Medical Research Council of Great Britain for a Junior Research Fellowship.

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Received July 9; revised August 20, 1974.

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### Erratum

In the article "Thegosis in herbivorous dinosaurs" by R. A. Thulborn (Nature, 250, 729; 1974) the seventh line in para 4 should read 'facets usually occur towards the cervix at the anterior or' . . . and not as printed.

In the article "TRH potentiates behavioural changes following increased brain 5-hydroxytryptamine accumulation in rats" by A. R. Green and D. G. Grahame-Smith (Nature, 251, 524; 1974) Figs 1 and 2 were transposed over their appropriate legends.

In the article "B and T-cell stimulatory activities of multiple mitogens from pokeweed" by M. J. Waxdal and T. Y. Basham (Nature, 251, 163; 1974) Figs 2 and 3 have been interchanged. The legends are correct as they stand.

# matters arising

### Taxonomy of the Taung skull

discussing the implications of new age estimates for the South African australopithecine cave localities1, Tobias2 suggests if, on further study, it becomes necessary to transfer the Taung skull to the robust lineage, the morphological immaturity of this specimen would preclude its being designated the type specimen for the robust lineage. He further suggests that since this transfer removes the holotype from the paradigm of Australopithecus africanus, a lectotype needs to be designated for this species in accordance with Article 74 of the International Code of Zoological Nomenclature. Both of these suggestions are made without basis. The morphological maturity of a specimen or its relative completeness have no bearing on its validity as a holotype. The application of Article 74 is only appropriate when no holotype was designated for a nominal species in the original publication and syntypes exist from which a lectotype can be chosen. Since the Taung skull is still in existence and Dart<sup>3</sup> designated no syntypes, Article 74 cannot apply.

It can be appreciated that a renaming of the robust lineage, A. africanus, would create confusion for the students of human evolution; however, it is one of the greatest strengths of the Code that it is more difficult to change the validity of a name assigned to a type specimen than it is to change an interpretation based upon the type specimen. Interpretations may change with the seasons but the identity of a type is eternal. The principal purpose of the Code is to produce stability, uniformity and clarity in the way scientific names are applied. In so doing, it does not attempt to regulate the usage of names in reference to species lineages. The Code only concerns the relationship of species names to type specimens and establishes which nominal species have available names and how the type specimen of each is determined. It is the responsibility of the scientist to determine where the type fits in the zoological world and its evolutionary history. The rules of taxonomy and the resulting stability between type specimens and species names do not restrict one's freedom to rearrange specimens in whatever phyletic scheme is warranted by the evidence; it only regulates which names can be validly

applied to the scheme. Tobias's apparent contention that the transfer of the Taung skull to another lineage removes it from being the holotype of A. africanus is totally without basis. Transfer of a holotype from one paradigm to another does not affect the association of the holotype with its designated name. The species name remains with the holotype not with the associated paradigm.

A clearer picture of this taxonomic manoeuvring is seen if it is considered that the Taung skull is not being transferred but rather all other specimens previously associated with A. africanus are being removed from this paradigm and all specimens assigned to the robust lineage are being transferred to A. africanus. Should all the gracile specimens be removed from A. africanus the next available name for this group would be A. transvaalensis Broom, 1936, the holotype being the Sterkfontein TM 1511 skull'. This name has priority over A. prometheus Dart, 1948'; Telanthropus capensis Broom and Robinson, 1949°; and Homo habilis Leakey, Tobias and Napier, 19647.

This would indeed create a confusing state of affairs and it may seem that following the Code in this case would produce a condition contrary to its stated purpose. The Code (Article 79) does sanction the suppression of a valid species name under extremely exceptional circumstances where the purposes of stability and uniformity are better served by suspending application of the Code. This is the only available procedure for suppressing the usage of A. africanus in reference to its type specimen.

Recent years have witnessed a significant improvement in the condition of hominid nomenclature. Three factors have been most responsible for this improvement: first, palaeontologists have ceased regarding every new fossil hominid as a new species; second, palaeontologists, zoologists and physical anthropologists have expanded the concept of variability in palaeospecies making it possible to lump greater ranges of morphological variation within a single species; and, third, the International Code of Zoological Nomenclature has been closely followed by most workers. If hominid nomenclature is to continue to be as parsimonious as possible then a strict adherence to the International Code

of Zoological Nomenclature is mandatory.

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PROFESSOR TOBIAS REPLIES-Olson has done palaeoanthropology a service in showing in what respects I misinterpreted the International Code Zoological Nomenclature, in the last three sentences of my article. Also, he has kindly and lucidly drawn attention to the intricacies of the correct nomenclatural procedures to be used. His comments on Taung are well taken, if one assumes the validity of (1) the original designation of A. africanus as the type-species of the new nominal genus Australopithecus (Articles 67-70 of the Code), and (2) the basing of the new nominal species on the Taung skull as its holotype (Articles 72-73).

If one assumes the procedures followed were valid, one is left with real problems which rigid adherence to the Code (except for Article 79) would in this instance bring in its wake, as Olson appreciates.

The crux may be stated as follows. The faunal evidence<sup>3,5</sup>, the estimated date of cave opening' and the sequence of geomorphological events, all point to a young age for Taung. If this age has been correctly inferred, it follows that either (1) Taung may represent a relict population of A. africanus; or (2) Taung does not represent A. africanus in the sense in which this taxon is understood from Makapansgat and Sterkfontein, a possibility suggested more than 5 years agos. (It does, of course, represent A. africanus, the original nominal species.)

If alternative (2) is correct, it would follow that Taung on the one hand, and Sterkfontein and Makapansgat on the other, may represent two different taxa.

In this event, I tentatively raised the possibility that the taxon represented by the Taung skull may be one of the robust taxa (for example, the super-

species, A. robustus/boisei). Indeed, fine morphological traits distinguish the Taung teeth from those of Sterkfontein and Makapansgat and, in a few instances, align them with those of the robust australopithecines7. If Taung proves to be robust, the confusing position set forth by Olson would follow. namely that the robust australopithecines would need to be added to the hypodigm of A. africanus, and the gracile australopithecines from Sterkfontein and Makapansgat would either remain as part of the hypodigm of a vastly more variable A. africanus, or need to be removed to form the hypodigm of another species (for which, as Olson points out, the nomen A. transvaalensis is available. Since there is now abundant evidence that the gracile and robust australopithecines are taxonomically distinct, the last-mentioned course would probably need to be adopted.

Such removal of the gracile australopithecines of Makapansgat and Sterkfontein from A. africanus would cause immense confusion, for the image of the species, A. africanus, is based largely on the hominids from these two sites, rather than on the skull of the Taung child, albeit the latter is the holotype.

No less of a muddle would ensue if palaeoanthropologists were now required to call the robust australopithecines A. africanus. It was to avert the resulting ambiguity that I raised the possibility of the holotype (the Taung skull) being removed from the paradigm of A. africanus—though I assumed and should have added, "if an appeal to the Commission under Article 79 of the Code were successful". In these unusual circumstances of extreme instability and confusion, not only is it reasonable to expect that such an appeal would be permissible under the Code (as Olson points out). but it might well be justified.

Meantime, the entire discussion has helped to establish the case for a comprehensive restudy of the Taung skull. Professor R. A. Dart, discoverer and nominator of the Taung skull, has generously invited me to undertake such a study.

P. V. TOBIAS

### Johannesburg

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### Paradox of Earth's core resolved

THE paradox of the Earth's core is hardly resolved by the recent comments of your Geomagnetism Correspondenti. Even your correspondent states that, because of uncertainties in the values of some of the physical parameters in the Earth's core, it is 'not possible to say whether convection in the core is feasible or not". He also states that "an origin (of the Earth's magnetic field), other than core convection, has never proved viable", and again "so sure are geomagneticians of the reality of core convection . . . It is certainly true that no completely satisfactory explanation has been given for the origin of the Earth's geomagnetic field. Most geophysicists, however, would support the idea that it arises from some kind of dynamo action in the core, but what drives the dynamo is very much a matter of contention. The two most reasonable proposals are thermal convection in the core and the Earth's precession; but in neither case has it been possible to establish that the mechanism would work.

Malkus<sup>2</sup> quoted experimental work and order of magnitude arguments to suggest that precession may produce turbulent motion in the core and thus drive the geodynamo. Such reasoning can, however, be seriously misleading, and contradictions can often result from over simplification. Malkus3 had earlier suggested that precessional torques may drive the geodynamo. Unfortunately, there are some errors in that article—the detailed theoretical investigation of a dynamo in a precessing turbulent core is extremely difficult and as yet no full treatment has been given. Rochester et al. (unpublished work) are working on this problem. At present it is impossible to reject precession as the driving mechanism for the geodynamo-it is as likely as thermal convection in the core.

Finally, your correspondent summarised the "flaw" in the Higgins-Kennedy argument that the core is stable against thermal convection'. That, however 'has already been pointed out (even more forcibly) by me' both in an earlier letter to Nature' and elsewhere6.

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### Blocking one-way maternal-foetal MLR

THE findings of Jones and Curzen¹ both that lymphocytes from most mothers had low response to stimulating related cord blood lymphocytes in mixed leukocytes reaction, and that only autologous maternal plasmas but not homologous plasmas had an inhibitory effect among those that had relatively high responses, are interesting. Despite the fact that the mixed lymphocyte reaction as measured after 6 d of incubation reflects the sensitisation and responsiveness in vitro rather than the state of previous sensitisation in vivo as demonstrated by us with the macrophage migration inhibition technique<sup>2-4</sup>, the results are consistent with our previous report3. It seems that the plasma blocking factors can also inhibit specific sensitisation in vitro of maternal cells as well as prevent the expression in vitro of cell mediated immune reactivities of lymphocytes previously sensitised (in vivo) to solubilised placental antigens as reported.

On the other hand, the findings of low maternal lymphocyte response to related foetal cells does not warrant the conclusion that maternal lymphocytes may somehow be affected by previous exposure to the plasma blocking factors. The results could as well be accounted for by the abnormality of the foetal cells in their effectiveness as stimulating cells. In fact, if previous exposure had any effect at all, the surface antigens of the foetal cells are a more likely target. The intact responsiveness of the maternal lymphocyte to stimulating related cord or unrelated adult lymphocytes in oneway mixed lymphocyte reaction was indicated by studies by Carr et al.3.

PORNCHAI MATANGKASOMBUT VIPADA YOUTANANUKORN Department of Microbiology, Mahidol University. Bangkok 4, Thailand

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cssential.

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(1653)

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### (1586

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### **Nutritional Adviser**

GLAXO-FARLEY FOODS, the foods division of Glaxo Laboratories Limited, requires a Company Nutritionist reporting to the Divisional Marketing Manager.

The position is located at the Plymouth Divisional headquarters and involves advising the Division generally on all nutritional ematters especially infant nutrition. This will include screening copy for sales packs and sales literature. He or she will help in the identification and composition of new products and will be responsible for arranging clinical trials of products and for maintaining appropriate professional contacts. Preparation of review articles, clinical papers and some lectures on nutrition will be necessary.

The position will involve some travel in the U.K. and will be both interesting and stimulating in a marketing orientated environment.

Applicants should be suitably qualified Nutritionists and/or dietitians with some previous experience.

We offer a competitive salary and excellent conditions of employment including bonus and pension schemes, and four weeks' holiday.



Please write for an application form, quoting ref. U 902 to the Personnel Officer (JSP), Glaxo Laboratories Limited, Greenford, Middlesex.

Scientific **Information Assistant** 

A young graduate in a biological science is required to join a team working on the organization of scientific information and new product registration in a research-based pharmaceutical company. The successful candidate will have an aptitude for the organization of scientific data, a wide interest in scientific and medical topics and will be able to write clear concise English. The vacancy provides a valuable introduction to information work for a graduate who does not wish to specialize.

Please apply to:

Mr C. E. Henry Staff Personnel Manager, John Wyeth & Brother Limited, Huntercombe Lane South, Taplow, Nr. Maidenhead, Berks. or Telephone: Slough 28311.

(1677)

### THE LONDON HOSPITAL

(WHITECHAPEL)

### MEDICAL PHYSICS TECHNICIAN

Applications are invited for the above post in the Heart Lung, Intensive Therapy Unit and Anaesthetic Research Unit of The London Hospital. The post will be both technical and scientific and will involve clinical measurement, blood gas analysis, gas analysis and the operation of Heart Lung equipment, i.e. pump, transducers, monitoring equipment and hyperthermia equipment.

Preference will be given to graduates in Biology and Physics but applicants with relevant 'A' levels in Biology or Physics may also apply. Opportunities will be available for further study.

Minimum starting salary will be £1,899 (increase pending) threshold payments.

For application forms, please ring or write to Moira Payne, Administrator Personnel Services, The London Hospital (Whitechapel), London E1 1BB, Tel. 01-247 5454 Ext. 388. (1649)

### CHELSEA COLLEGE University of London **ENVIRONMENTAL** MONITORING PROJECT

Applications are invited for the following research Applications are invited for the following research posts associated with a three year study, sponsored by the Department of the Environment, on the collection, collation, manipulation and assessment of data which will flow from existing and planned monitoring activities at national, regional and global levels:

global levels:

ONE ENVIRONMENTAL SCIENTIST
(Salary range £2,580 to £5,976)\*

ONE SYSTEMS ANALYST
(Salary range £2,580 to £5,976)\*

ONE RESEARCH ASSISTANT IN
ENVIRONMENTAL SCIENCE
(Salary range £1,809 to £2,580)\*

ONE RESEARCH ASSISTANT IN
SYSTEMS ANALYSIS
(Salary range £1,809 to £2,580)\*

\* In addition a London Allowance of £213 per annum and Threshold Payments will be made.
Where appropriate Secondment may be arranged.
The programme is designed to:
(a) Review the current status of environmental

(a) Review the current status of environmental problems and monitoring practices.
 (b) Define needs and goals in the near future.

(c) Determine the most efficient procedures for realising these objectives.

The team will be directed by Professor G. T. Goodman, Chelsea College, Application forms and further particulars from the Personnel Officer, Chelsea College (N), Manresa Road, London SW3 6LX. Closing date for receipt of applications November 14, 1974. (1700)

### UNIVERSITY COLLEGE DUBLIN APPOINTMENT IN CHEMISTRY

APPOINTMENT IN CHEMISTRY

Applications are invited for a teaching post in the Department of Chemistry, Candidates should have a special interest in Physical Chemistry.

The current salary scales are:
College Lecturer: £3,514 by 162 to £4,648
Assistant Lecturer: £2,420 by 129 to £3,323
Entry point on the relevant scale will be in accordance with qualifications and experience.
A non-contributory pension scheme and family allowances are additional to salary. An alternative contributory F.S.S.U. type pension scheme is also available.

Prior to application, further information (including application procedure) should be obtained

avanable.

Prior to application, further information (including application procedure) should be obtained

Mr. J. P. MacHale, Secretary and Bur University College, Belfield, Dublin 4.

Telephone 693244 Extn. 431.
Latest date for receipt of completed applications riday November 22, 1974. (1656)

### **BIOCHEMIST**

Ph.D. or equivalent. Must have laboratory experience in vitamin D chemistry or action. To establish a vitamin D-focused laboratory programme in cona vitamin D-focused laboratory programme in conjunction with university calcium researchers. Position at Research Institute. The Hospital for Sick
Children, Toronto, with faculty appointment in
Department of Biochemistry, University of Toronto.
Salary commensurate with experience and qualifications. Apply before December 1st enclosing
curriculum vitae and names of two referees, to:—
The Secretary, Research Institute, The Hospital for
Sick Children, 555 University Avenue, Toronto,
Ontario, M5G 1X8, Canado. (1661)

### ASSISTANT PROFESSOR WILDLIFE BIOLOGY

Position available as a joint appointment in Institute of Animal Resource Ecology and Department of Zoology beginning in July 1975 for one person to develop an integrated program in wildlife studies and resource management. Candidate must have ability to do quantitative field studies on larger mammals or birds and to direct graduate work on wildlife species. Teaching will involve one undergraduate course in ecology or behaviour and a graduate course in area of specialization.

Curriculum vitae and names of three referees should be sent to Dr. W. G. Wellington, Director, Institute of Animal Resource Ecology, University of British Columbia, Vancouver, Canada V6T 1W5. Closing date: January 31, 1975. (1665)

### **BIOCHEMICAL JOURNAL EDITORIAL ASSISTANT**

required. Duties consist mainly in preparing papers for the printer and proof-reading for the Biochemical Journal and other Biochemical Society publications. A good degree in biochemistry or related subject is essential. Some postgraduate related subject is essential. Some postgraduate experience in laboratory and/or editorial work is desirable, though not essential. The commencing salary will be in the incremental range £2,331 to £2,625 per annum. Please apply in writing, giving the names of two referees, to the Editorial Secretary, Biochemical Journal, 7 Warwick Court, London WCIR 5DP. (1691)

# CHEMIST or BIOCHEMIST

Graduate chemist or biochemist to study the metabolism of foreign compounds. Several years of relevant experience is essential.

# MICROBIOLOGIST

Graduate microbiologist to join a small team using organ and tissue culture in toxicology and carcinogenesis studies.

# ANALYTICAL CHEMIST

To develop methods for the analysis of drugs in biological fluids.

Competitive salaries, non-contributory pensions and free life assurance are offered by this rapidly-expanding contract research company. situated in beautiful rural surroundings, 40 minutes from London.

Please send your curriculum vitae to: J. W. Daniel, D.Sc., Director of Metabolic Studies.

LIFE SCIENCE RESEARCH Stock, Essex CM4 9PE



(1692)

# nature

A vacancy will arise soon in the Washington office of *Nature* for the important post of Assistant Editor responsible for supervising the refereeing of biological manuscripts submitted to the Washington office. A period of training in the London office under the supervision of the Biological Manuscripts Editor will precede the posting to Washington. The successful applicant may expect to be in Washington for not less than two years.

This job is an outstanding opportunity for a recently qualified British or American Ph.D who has a strong interest in ensuring the high quality of the biological papers we publish.

Apply with curriculum vitae and the names of two referees to:—

The Editor. Nature, Macmillan Journals Ltd., 4 Little Essex Street. London, WC2R 3LF

by November 15th

### **British Museum (Natural History)**

Study the variations exhibited by molluscan faunas of freshwater habitats 
Undertake curatorial and advisory work on land and freshwater molluscs.

☐ Degree, preferably 1st/2nd hons or equivalent in Zoology ☐ Keen interest in evolutionary biology and taxonomy ☐ Experience of techniques for examining biochemical variation an advantage ☐ Age under 27 ☐ Appointment as

Scientific Officer (over £2150 to around £3250) 

Ref: SB/36/DK.

Application forms (for return by 22 November 1974) from Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, telephone Basingstoke 29222 ext. 500 (or, for 24 hour answering service, London 01-839 1992).

> **Ministry of Overseas Development** Tropical Products Institute, Slough

# **Crop Storage Engineers**

R & D and advisory work on structures and systems for handling and storage of cereal grains and other crops in developing countries ■ Assist in training of storage technologists from overseas ■ Overseas assignments may be required.

Degree or equivalent in Agricultural, Mechanical or Civil Engineering Knowledge of simple structural design grain storage systems desirable ☐ Experience in tropical or sub-tropical countries an advantage ☐ Age under 30 ☐ Appointment as Scientific Officer (around £1750 – over £2800) or Higher Scientific Officer (£2600 - over £3500), according to age and experience Ref: SA/35/JD.

☐ Application forms (for return by 15 November 1974), from Miss C A Hall, Tropical Products Institute, 127 Clerkenwell Road, London EC1R 5DB.

Ministry of Agriculture, Fisheries and Food Food Science Division, Norwich

■ (Ref: SB/13/AE) To lead team investigating reactions of nitrite with food components 
Study rates and products of C-, S- and N-nitrosations in meat 
Relate results to nitrite losses during meat processing, distribution and culinary preparation Develop analytical methods for measuring nitrite products.

☐ 1st/2nd hons degree in Chemistry or related subject ☐ At least 2 years' relevant postgraduate experience, preferably including analysis of trace compounds in complex media  $\square$  A working knowledge of reaction kinetics in biological systems an advantage  $\square$  Age under 30  $\square$  Appointment as Higher

Scientific Officer (£2600 – over £3500).

(Ref: SB/14/AE) In team investigating reactions of nitrites in meat 
Study S-nitroso compounds as nitrosating agents and as a nitrite 'sink' 
Reflected findings to nitrite loss in meat and compounds present in food eaten.

☐ Ist/2nd hons degree in Chemistry or related subject ☐ Interest in analytical and kinetic aspects of organic chemistry in systems of biological origin desirable  $\square$  Age under 27  $\square$  Appointment as Scientific Officer (around £1750 –

Application forms (for return by 19 November 1974), from Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, telephone Basingstoke 29222 ext. 500 (or, for 24 hour answering service London



(1724)

### UNIVERSITY OF SURREY DEPARTMENT OF BIOCHEMISTRY

Research Officer required to work in radio-immunoassay in relation to disease in man. Pre-vious experience desirable but not essential. Salary in the region £1,593 to £2,229, plus Threshold pay-ments, depending on experience.

Write giving curriculum vitae to Professor Vin-cent Marks, Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH. (1658)

### ROYAL HOLLOWAY COLLEGE, (UNIVERSITY OF LONDON),

Egham Hill, Egham, Surrey.

Applications are invited for a Research Assistant to work on enzyme studies in tropical fruit. Candidates should have a good Honours degree in Biochemistry or Chemistry. There is the possibility of registering for a higher degree of the University of London.

Please apply to Professor J. B. Pridham, Department of Biochemistry. (1668)

### MOLECULAR BIOLOGIST

MOLECULAR BIOLOGIST
The National Institute of Environmental Health Sciences invites applications for a Molecular Biologist position under the Staff Fellow or Visiting Associate Programme. As a member of a multidisciplinary group studying developmental toxicology, the selected applicant will plan and conduct independent and collaborative research in the area of the molecular basis for chemically-induced alterations in developing systems.

A doctorate in Biochemistry or a related disippline is required.

The appointment will initially be for one or two years and may be extended. Salary will range from \$15,000 to \$20,000 per annum depending on qualifications.

Letters of application with curriculum vitae, bibliography and name and address of two references should be addressed to:

Mr Charles E. Walker

National Institute of Environmental

Health Sciences

P.O. Box 12233

Research Triangle Park,

North Carolina 27709

Letters must be received by December 10, 1974 to be considered.

An Equal Opportunity Employer (1715)

to be considered. An Equal Opportunity Employer (1715)

### MEDICAL RESEARCH COUNCIL NATIONAL INSTITUTE FOR MEDICAL RESEARCH

### DIVISION OF VIROLOGY

Applications are invited from young postdoctoral virologists for a position within a group concerned with basic mechanisms of virus infection using the adenovirus model system. This post should be of interest to those who have experience of any branch of molecular biology. The appointment will be for up to three years and will be in the salary range £2.412 to £3.636 p.a. according to age and qualifications (plus London and threshold allowances). Superannuation provision under F.S.S.U. Applications, giving details of qualifications, experience and the names of two professional referees, should be sent to the Director. National Institute for Medical Research, Mill Hill, London NW7 1AA before December 6, 1974. (1666)

### UCLA

UCLA

University of California, Los Angeles, Department of Geology invites applications for two-year faculty appointments as assistant professor. A Ph.D. is required. Two faculty two-year positions will be available beginning September 1975. Applicants must have truly superior capabilities in one or more of the relevant basic physical or biological sciences. Two earth scientists will be selected from the three following general areas:

(1) Those engaged in research in one or more of the fields (a) igneous or metamorphic petrology; (b) ore genesis: (c) experimental or theoretical geochemistry; (d) mineral physics. Examples of emphasis include thermodynamics of irreversible processes and chemical or thermal diffusion.

(2) Those engaged in research in the areas of tectonophysics, geological physics, or structural geology. Of particular interest are: (a) solid-state aspects of microscopic phenomena-fracture and flow of rocks, diffusion, thermodynamics, and lattice dynamics; (b) global tectonics; (c) quantitative modelling of large or small scale processes.

(3) Marine science, those engaged in research in organic geochemistry, ecology, or marine geology. Examples of emphasis include diagenesis of sediments, interstitial water, evolution, phycology (calcarcous algae), invertebrate biology, and global tectonics.

Send vitae and two letters of reference to:

(cafeareous aigae), invertebrate 0000/gs, and global tectonics.

Send vitae and two letters of reference to:
Clarence A, Hall, Chairman
Department of Geology
University of California Los Angeles
Los Angeles, California 90024

Minority and ethnic earth scientists are encouraged to apply. UCLA is an affirmative-action/equal opportunity employer. (1654)

### **ELECTRON MICROSCOPY** TECHNICIAN

required with experience in Electron Microscopy to collaborate on a project involving study of Tumour Virus DNA.

Salary according to qualifications and experience. Write or telephone for application form to Miss S. M. Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields, W.C.2. on 242 0200 ext. 305. (1716)

### KENNEDY INSTITUTE OF RHEUMATOLOGY CHIEF TECHNICIAN

required for the Division of Experimental Pathology. The work involved is mainly of a research nature and histology autoradiography, and electron microscopy services are provided for other divisions in the Institute.

sions in the Institute.

This is a well equipped, modern laboratory investigating lymphoid tissue function. All the usual techniques required to study cells and tissues are employed. A suitable candidate would be expected to have the relevant background experience as well as to be prepared to take on administrative responsibilities. Written applications giving full curriculum vitae and naming two referees to: Laboratory Superintendent, Kennedy Institute of Rheumatology, Bute Gardens, London W6 7DW.

(1507)

(1507)

### INFORMATION SCIENTIST

Borax Consolidated Limited, an International company within the R.T.Z. Group, requires an Information Scientist for the Information Department at its Research Centre in Chessington, Surrey. The information team is responsible for providing the Borax companies throughout the world with technical, patents, and commercial information.

technical, patents, and commercial information. Candidates should have a degree (or equivalent qualification) in chemistry or a related field Previous experience in information work is desirable and fluent reading ability in one of more modern languages will be a distinct advantage. Every opportunity will be given to the successful candidate for fullest utilisation of ability and initiative. The post offers an attractive starting salary and excellent fringe benefits, including a non-contributory pension scheme.

Please apply for an application form to: The

Please apply for an application form to:—The Personnel Manager, Borax Consolidated Limited, Borax House, Carlisle Place, London SWIP 1HT. Tel: 01-834 9070. (1659)

### WEST BERKSHIRE HEALTH DISTRICT MEDICAL PHYSICS TECHNICIAN

for the modern Isotope Laboratory at the Royal Berkshire Hospital, Reading for interesting work in the chemistry and physics of medical isotope techniques. Post will be on Grade IV £1,773 to £2,463 plus additional threshold payments. Day release for further training possible. (Normally ONC or 2 'A' levels required).

Reading is a pleasant University town offering easy access to London, Oxford, Windsor, Henley and attractive Thames country-

written applications with details and naming 2 referees to: Hospital Secretary, Royal Berkshire Hospital, London Road, Reading, Berkshire. (1720)

### GRADUATE RESEARCH ASSISTANT

required in the Department of Nuclear Medicine for work involving the quantitation of radioisotope images using two Gamma cameras interfaced with a mini computer. Mathematics and experience in computer programming essential.

Salary in range £1,758 to £2,022.

Applications to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. N.M., enclosing a curriculum vitae and the names of two referees.

### SURREY EDUCATION COMMITTEE EWELL COUNTY TECHNICAL COLLEGE

Reigate Road, Ewell, Surrey Principal: B. Haynes BSc DipEd FRIC DEPARTMENT OF BIOLOGICAL SCIENCES

Require as soon as possible:

LECTURER I to teach Cell Biology and/or Biochemistry for H.N.C. in Medical Laboratory Subjects. The College is an approved centre for this course. Some experience of Medical Microbiology or Histopathology and an interest in research on problems of learning in the bio-medical field would be advantageous.

Applicants with suitable experience will have the opportunity to teach MIBiol (Hons, BSc.) level. Salary: £1,800 to £2,874 p.a. For Gd Hons, Grad, the scale is extended to £3,045 p.a. £141 p.a. London Allowance payable, (Subject

to confirmation)

Generous relocation expenses and assistance with house purchase in approved cases.

Stamped addressed foolscap envelope please for further particulars and application form from the Vice-Principal. Applications to be returned within 14 days. (1674)

# CSIRO

### **AUSTRALIA**

# DIVISION OF TROPICAL **AGRONOMY**

# KIMBERLEY RESEARCH STATION KUNUNURRA, W.A.

# RESEARCH SCIENTIST

The Commonwealth Scientific and Industrial Research Organization has a broad charter for research into primary and secondary industry areas. The Organization has approximately 6,500 employees—2,100 of whom are research and professional scientists-located in Divisions and Sections throughout Australia.

# GRAIN SORGHUM BREEDER

GENERAL: Kimberley Research Station is a unit of the Division of Tropical Agronomy, which has its headquarters at Brisbane. The Station is situated at Kununurra in the Ord River valley in the north of Western Australia, and is engaged in research into pastures and crops suitable for the tropical latitudes of Australia. The township of Kununurra services the Ord River Irrigation Area and has a population of about 2,000.

Because of its latitude (16°S) and climate, and excellent field and irrigation facilities, the Kimberley Station is an ideal location for research on tropical sorghums. Breeding of grain sorghum was commenced at the Station in 1970 and considerable progress has been made in developing better-adapted genotypes for the tropical environments of northern Australia.

DUTIES: CSIRO is now seeking a Sorghum Breeder to continue this program. and to collaborate in new studies on physiological factors limiting grain sorghum yields in tropical Australia.

QUALIFICATIONS: A degree in Agriculture or Science, and a Ph.D. or equivalent postgraduate experience in plant genetics, supported by satisfactory evidence of research ability. Experience in plant breeding is essential.

SALARY: Depending upon qualifications and experience, the appointment will be made within the salary range of Senior Research Scientist, \$A13,424 to \$A15,456 p.a. or Principal Research Scientist, \$A16,060 to \$A18,299 p.a. plus local allowances of not less than \$A1,310 (married) or \$A930 p.a. (single).

CONDITIONS: This position is available for an indefinite period and Australian Government Superannuation benefits are available.

Six weeks' recreation leave per annum will be granted and this leave may be accumulated for three consecutive years. Fares for travel to and from Perth or equivalent travelling will be provided by the Organization once each two-year period. The area is classified Zone A for taxation purposes.

A house with basic furniture is available for rental.

Applications stating full personal and professional details, the names of at least two professional referees and quoting Reference Number: 620/76 should reach:-

The Personnel Officer, Australian Scientific Liaison Office, 64-78, Kingsway, LONDOŇ WC2B 6BD

by the 29th November, 1974.

Applications in U.S.A. and Canada should be sent to The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W., WASHINGTON, D.C. 20036, U.S.A.

(1721)

## .CSIRO

# **AUSTRALIA**

# DIVISION OF COMPUTING RESEARCH

CANBERRA, A.C.T.

# RESEARCH SCIENTIST

The Commonwealth Scientific and Industrial Research Organization has a broad charter for research into primary and secondary industry areas. The Organization has approximately 6,500 employees-2,100 of whom are research and professional scientists-located in Divisions and Sections throughout Australia.

# **OPERATING SYSTEMS**

**GENERAL:** The Division of Computing Research has a Control Data Cyber 76 Computer (commonly called a 7600) in Canberra. With processor capable of averaging fifteen million instructions per second, central memory exceeding three million characters, disc capacity of twelve hundre dmillion characters, and with substantial expansion capacity, the configuration provides an unsurpassed tool for research and development projects.

The Cyber 76 computer is currently linked to a network of batch terminals by a Control Data 3600 Computer. This will be replaced by a pair of CONTROL DATA model 172 computers, the first of which is scheduled for delivery early in 1975. These "front-end" computers will provide operator control functions, magnetic tape handling facilities and drive the terminal network. It is hoped to evolve a system capable of handling a large number of interactive consoles and batch terminals, with little degradation of source in case of a single CYRER 173 follows. terminals, with little degradation of service in case of a single CYBER 172 failure.

A rapidly expanding network of mini computers at CSIRO research centres is linked by Australian Post Office data services and enables the concentration of interactive and batch work in Canberra. Currently, about thirty batch terminals and about two hundred interactive terminals are connected.

DUTIES: To work as part of a team engaged in research, development and maintenance of operating system software for both the Cyber 76 and the new front-end computers. Members of the team are expected to take some responsibility for day-to-day system maintenance but will also need the ability to undertake original and major projects.

Areas of likely development include the provision of interactive access to the Cyber 76 and front-end computers, job scheduling, archival file storage and connection to communication networks.

**QUALIFICATIONS:** A Ph.D. degree (or equivalent) in computing science, together with demonstrable research ability is essential and previous experience in operating systems work is desirable.

**SALARY:** Appointment will be made within the salary ranges of Research Scientist or Senior Research Scientist: \$A10,448-\$A15,456 p.a.

TENURE: This position is available for an indefinite period and Australian Government Superannuation benefits are available.

Applications stating full personal and professional details, the names of at least professional referees, and quoting Reference Number: 900/248, should

The Personnel Officer Australian Scientific Liaison Office, 64-78, Kingsway, LONDON, WC2B 6BD

by the 29th November, 1974. Applications in U.S.A. and Canada should be sent to The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W., WASHINGTON, D.C. 20036, U.S.A.

(1722)

### TEESSIDE **POLYTECHNIC**

Applications are invited for a post of Research Assistant in The Department of Mathematics and Statistics, to carry out research into multistep methods of numerical integration of differential equations.

Applicants must have, or expect to obtain, a good honours degree in mathematics.

The post will be for a minimum of two years at a salary of £1,500 per annum.

Further details and application forms may be obtained from the Staffing Section Teesside Polytechnic, Borough Road, Middlesbrough, Cleveland County TS1 3BA.

(1745)

### JAMES COOK UNIVERSITY OF NORTH QUEENSLAND LECTURER IN BOTANY

Candidates should have a special interest and research experience in some aspect of Plant Genetics although applications will also be considered from those having an interest in some related field provided they are competent to conduct undergraduate courses in Plant Genetics. The appointee will be expected to assist in the general teaching and research activities of the Department. Salary range: \$A9,002 to \$12,352 p.a. plus a locality allowance of \$A142 p.a. for a married male or \$A71 p.a. for a single appointee. Conditions of appointment include F.S.S.U. superannuation, invalid pension scheme, housing assistance, study leave and allowance for travel and removal expenses on appointment. Further details and application forms obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WCIH OPF.

Applications close November 29, 1974. (1669)

### UNIVERSITY OF WALES WELSH PLANT BREEDING STATION

WELSH PLANT BREEDING STATION

Applications are invited for a Scientific Officer support post in the Chemistry Department of the Station to undertake and supervise non-routine analytical work associated with the research programmes in cereal and herbage chemistry.

The qualification required would be a Higher National Certificate in Chemistry or an equivalent qualification, with experience and interest in the use of automated analytical equipment including amino acid analysers.

Salary scale £1,592 to £2,675 per annum plus threshold agreement at present £2.80 per week. There is a non-contributory superannuation scheme and male employees are required to contribute 1½% of salary to the Widows' and Chidren's Pension Scheme.

Commencing salary will be determined by age

Commencing salary will be determined by age

and experience.

Applications by letter only, together with names of two referees, to be sent to the Secretary, Welsh Plant Breeding Station, Plas Gogerddan, Nr. Aberystwyth, Dyfed SY23 3EB on or before November 15, 1974.

### UNIVERSITY OF EDINBURGH EDINBURGH SCHOOL OF AGRICULTURE

### LECTURER IN TROPICAL ANIMAL PRODUCTION

ANIMAL PRODUCTION

Applications are invited for a short-term appointment (3 ot 4 years) of Lecturer in Tropical Animal Production.

The successful candidate will contribute to a new course in Tropical Animal Health and Production by the Centre for Tropical Veterinary Medicine and the School of Agriculture.

Agriculture.

Salary on the scale £2,118 to £4,896 per annum. Superannuation under F.S.S.U.,

Applications should be sent to the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL, from whom further particulars may be obtained. Please quote reference 1042.

M42. The closing date is November 25, 1974. (1736)

### FOURAH BAY COLLEGE UNIVERSITY OF SIERRA LEONE

UNIVERSITY OF SIERRA LEONE
Applications are invited for the post of PROFESSOR AND HEAD OF THE GEOGRAPHY
DEPARTMENT, tenable September 1, 1975. Salary
scale (under review): Le6,000 to Le6,800 p.a.
(Le2=£1 sterling). The British Government may
supplement salary by £2,850 (sterling) p.a. for a
married appointee and £1,854 (sterling) p.a. for a
single appointee (normally free of all tax) and
provide children's education allowances and holiday visit passages. £5.5.U. Family passages;
various allowances; regular overseas leave. Detailed
applications (2 copies), including a curriculum
vitae and naming 3 referees, should be sent by
airmail, not later than November 27, 1974, to the
Secretary University of Sierra Leone. Applicants resident
in U.K. should also send 1 copy to Inter-University
Council; 30/91 Tottenham Court Road, London
WIP ODT. Further particulars may be obtained
from either address. (1679)

### UNIVERSITY OF ZAMBIA

UNIVERSITY OF ZAMBIA

Applications are invited for (a) LECTURESHIP or (b) ASSISTANT LECTURESHIP IN PLANT SCIENCE, School of Agricultural Sciences. Applicants should possess M.Sc. and/or Ph.D. in Agronomy or Crop Physiology, and have teaching and/or research experience in these fields. Appointe will be required to teach the basic courses in Plant Science concerned with tropical pastures, forage crops and other fundamentals of Crop Production. Salary scales: (a) K4,600 to K5,400 p.a. (b) K3,200 to K3,800 p.a. (£1 sterling=K1.50). The British Government may supplement salaries of married appointees at Lecturer level in range £516 to £666 p.a. (sterling) (normally free of all tax) and provide children's education allowances and holiday visit passages. This supplementation is unlikely to apply to single appointees at Lecturer level. Family passages; various alllowances; superannuation and medical aid schemes; regular overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by air mail, not later than November 27, 1974, to the Registrar, University of Zambia. P.O. Box 2379, Lusaka, Zambia. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London WIP 0DT. Further particulars may be obtained from either address. (1678)

### UNIVERSITY OF NOTTINGHAM PHYSICS POSTDOCTORAL RESEARCH

ASSISTANT

is required to work on the propagation of very high frequency phonons in solids and liquid helium at 0.1K. Techniques include superconducting motors to move phonon sources and tunnel junction detectors at dilution refrigerator temperatures. Manual dexterity, stamina and physical insight are more important than detailed experience. Salary £2,247 plus threshold.

Applications to Dr A, F. G. Wyatt, Department of Physics, University of Nottingham, University Park, Nottingham NG7 2RD. (1684)

SECTION OF MOLECULAR PHARMACOLOGY

of the UNIVERSITY OF MAINZ GERMANY

RESEARCH ASSOCIATES (Ph D in Biochemistry, Organic Chemistry or Cell Biology)

and TECHNICIANS WITH

MASTERS DEGREE (Biochemist, Organic Chemist or Cell Biologist)

required for well-equipped research laboratory to work on mechanisms of chemical carcino-involving synthetic and/or biochemical work, animal experimentation and/or tissue culture.

or tissue culture.

Salary within the range of £5,000 to £8,000 per annum for the research associate, £3,200 to £6,500 for the technician.

Start of appointment December 1, 1974 or later (some openings April 1, 1975).

Experience in solubilization, purification and study of enzymes and knowledge of German an advantage but not a prerequisite. Detailed applications with the names of referees and giving relevant laboratory experience should be sent to Dr F. Oesch, Biozentrum of the University, Klingelbergstrasse 70, CH-4056 Basel, Switzerland.

# BIOCHEMIST or CELL BIOLOGIST, **SWEDEN**

Pharmacia Fine Chemicals AB, manufacturers of SephadexR and other products for biochemical separations, require a biochemist or cell biologist to work at our headquarters in Uppsala, Sweden.

The successful applicant will join our Scientific Information and Technical Services group. The activities of the group include

- \* the preparation of all kinds of scientific information material
- applications work and laboratory testing of new products
- \* lecturing

but applicants should be prepared for extremely varied work and certain amount of travelling, mostly within Europe.

Applicants should have research experience in biochemistry or cell biology. Clear and accurate expression in the written word is essential with English as the Mother tongue.

This is an important position and carries an appropriate salary. Preferred age 25 to 30.

Write in confidence to:

Mr D. Sweetman Pharmacia (Great Britain) Ltd **Paramount House** 75 Uxbridge Road London W5 5SS



### HAZLETON LABORATORIES EUROPE LIMITED

require a

### BIOCHEMIST or BIOCHEMICAL PHARMACOLOGIST

preferably a Ph.D. with some experience in drug metabolism and/or the use of isolated organ perfusion techniques, to join a team which is studying factors affecting foreign compound disposition and metabolism. The principal area of interest, at present, is tobacco smoke but the scope is expected to extend rapidly into other fields.

Excellent facilities for research are available in well-equipped laboratories set in pleasant rural, but accessible surroundings.

Please write, giving brief details of qualifications and experience to:

The Personnel Officer, (Ref. R.O.e), Hazleton Laboratories Europe Limited,

Harlow Hill,

HARROGATE HG3 1PY.

(1688)

### LEICESTER POLYTECHNIC

SCHOOL OF CHEMISTRY

Applications are invited for the following research ssistantships:

(1) Bio-inorganic chemistry; for studies on the drug penicillamine in relation to Wilson's Disease.

drug penicillamine in relation to Wilson's Disease.

(2) Solid state chemistry, for studies of mass transport in ionic solids.

Applicants should have a good honours degree in Chemistry or equivalent qualification.

For post (1) an interest in analytical inorganic chemistry and/or protein chemistry is necessary. Some experience in one of these fields would be an advantage.

Post (2) requires a competent experimentalist with an interest in radiotracer techniques and/or crystal growth.

Appointments are for three years and commencing salary is £1,824 p.a. Successful applicants holding a Bachelors or Masters degree will be expected to register for a Ph.D.

Intending applicants are encouraged to phone Roger Linford (0533 50181 ext. 2202) for further particulars. Application forms are obtainable from Staffing Officer, Leicester Polytechnic, P.O. Box 143, Leicester LE1 9BH. (1689)

# **AUSTRALIA Public Service of Victoria Ministry for Conservation** PROJECT DIRECTOR

(THREE POSITIONS)

Position No. 1—PORT PHILLIP BAY. REF. No. (H/07) Position No. 2—WESTERNPORT BAY. REF. No. (H/08) Position No. 3—GIPPSLAND LAKES. REF. No. (H/09)

Yearly Salary \$A16,393—SCIENTIFIC OFFICER **\$A16,207—ENGINEER** 

### **Duties:**

Subject to the Director of Environmental Studies, to be responsible for the general direction and co-ordination of the Port Phillip Bay, Westernport Bay and Gippsland Lakes Environmental Studies. To maintain liaison with, and co-ordinate, the activities of the Government, University and other groups participating in such Study. To summarise and report on activities of participating groups and to assist in the application of research activities to environmental management.

### Qualifications:

An approved degree in the physical or biological sciences, or an approved degree in engineering or other qualification admitting to Membership in the Institution of Engineers, Australia, or other appropriate qualification; a higher degree desirable. Demonstrated ability in working in multi-disciplinary groups and proven ability at a senior level to plan, develop and lead inter-disciplinary investigations. Experience in the practical application of research findings to management and a knowledge of the working of Government, University and Industry desirable. Experience in a marine science an advantage.

Separate applications must be submitted for these positions.

Applications quoting appropriate reference number should be addressed to the Secretary, Public Service Board of Victoria, State Public Offices, No. 1 Treasury Place, Melbourne, 3002, Australia, by not later than 9.30 a.m. on Wednesday, November 20, 1974, together with statements of experience and qualifications and date and place of birth. (1742)

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG, SOUTH AFRICA DEPARTMENT OF ANATOMY SENIOR LECTURESHIP IN **HUMAN ANATOMY** 

HUMAN ANATOMY

Applications are invited for appointment to the post of Senior Lecturer in the Gross Anatomy section of the Department of Anatomy, Medical School, Candidates must be capable of teaching General Anatomy, Micro-Anatomy and Living Anatomy to Medical, B.Sc., Physiotherapy and Occupational Therapy, and Nursing students. The possession of a Ph.D., or other higher degree, with or without a medical qualification, is essential. Salary will be determined according to qualifications and experience, and presently falls within the following ranges

Senior Lecturer (2) R10,350 to R11,730. Senior Lecturer (3) R9,315 to R10,695.

These scales are to be improved shortly.

Benefits include an annual bonus, pension and medical aid facilities, and a housing subsidy, if eligible.

Intending applicants should obtain the information sheet relating to the post from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa, with whom applications should be lodged not later than November 30, 1974. U.K. applicants may obtain the information sheet from the London Representative, University of the Witwatersrand, 278 High Holborn, London, W.C.I to whom a copy of the application should be sent.

(1682)

### St. Mary's Hospital Medical School Paddington, London W2 1PG

# RESEARCH ASSISTANT

Graduate or chartered engineer with practical experience in Electronics or Applied Physics, and preferably in Bio-Engineering required. Work, supervised jointly by Dept. of Obstetrics and Gynaecology, and Dept. of Bio-Engineering will mainly be involved in development of new techniques for surveillance of foetus in pregnancy and labour. Initial appointment for 3 years,

Appropriately qualified applicants may read for higher degree in Biomedical Engineering. Commencing salary within scale £2,064 to £2,325 p.a. plus £213 L.A. plus threshold. Further details from Dr. R. E. Trotman. Apply, The Secretary.

# **AUSTRALIA Public Service of Victoria Ministry for Conservation Fisheries and Wildlife Division** PROJECT LEADER

(THREE POSITIONS)

Position No. 1—WESTERNPORT. REF. No. (H/04)

Position No. 2—GIPPSLAND LAKES. REF. No. (H/05)

Position No. 3—PORT PHILLIP BAY. REF. No. (H/06)

Yearly Salary \$A14,520

### **Duties:**

To be responsible for the supervision and direction of research activities conducted by the Marine Pollution Studies Section in the Westernport Bay, Gippsland Lakes and Port Phillip Bay Environmental Studies. To assist in the co-ordination of research activities and to maintain liaison with other groups participating in environmental investigations. To prepare reports and recommendations. Other duties as directed.

### **Qualifications:**

An approved degree in Science or an equivalent qualification; preferably a higher degree. Demonstrated ability and extensive

experience in marine ecological research and in the supervision of scientific staff. To be capable of working with multi-disciplinary groups and to have report writing experience.

### Note:

Separate applications must be submitted for these positions.

Applications quoting appropriate reference number should be addressed to the Secretary, Public Service Board of Victoria, State Public Offices, No. 1 Treasury Place, Melbourne, 3002, Australia, by not later than 9.30 a.m. on Wednesday, November 20, 1974, together with statements of experience and qualifications' and date and place of birth.

### QUEENSLAND INSTITUTE OF TECHNOLOGY DEPARTMENT OF CHEMISTRY TECHNOLOGIST DIVISION II QUOTE: V.106/74

SALARY RANGE: \$7,496 to \$10,637 (AUST.) p.a.

MINIMUM QUALIFICATIONS:

(a) A degree in science, applied science or chemical engineering from a University or college of advanced education; or equivalent formal tertiary qualifications.

(b) Experience and skills sufficient to apply advanced knowledge.

EXPERIENCE REQUIRED:

(a) A minimum of five years' technical experience which has been gained in scientific and/or technological laboratories.

An ability to co-operate and work efficiently with other staff.

DUTIES: One or more of the following:

(a) Assist in the development of new experiments for application to the Department's teaching programme, as well as updating existing experiments when required. The teaching programme encompasses inorganic and analytical chemistry, organic chemistry, physical chemistry and chemical engineering/chemical technology.

(b) Provide technical guidance and assistance to staff and students on the operation of equipment associated with the teaching programme.
 (c) Assist in departmental research and service projects.

(d) Prepare reports and recommendations on the above.

Applications quoting position, reference number, and containing full particulars of name, address, telephone number, date of birth, marital status, qualifications, experience and present employment and the names and addresses of at least two referees, should be forwarded to the Registrar, Queensland Institute of Technology, Box 246, P.O., North Quay, Brisbane, Queensland, Australia, by Saturday November 23, 1974. (1723)

### MEDICAL RESEARCH COUNCIL NATIONAL INSTITUTE FOR MEDICAL RESEARCH DIVISION OF VIROLOGY

Applications are invited from 'young postdecotoral virologists for a position within a groupconcerned with basic mechanisms of virus
infection using the adenovirus model system. This
post should be of interest to those who have
experience of any branch of molecular biology.
The appointment will be for up to three years
and will be in the salary range £2,412 to £3,636
p.a. according to age and qualifications (plus
London and threshold allowances). Superannuation provision under F.S.S.U. Consideration will
be given to those who may not be available until
the autumn of 1975.

Applications, giving details of qualifications.

the autumn of 1975.

Applications, giving details of qualifications, experience and the names of two professional referees, should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA before December 6, 1974. (1699)

UNIVERSITY OF READING DEPARTMENT OF AGRICULTURE AND HORTICULTURE

### ASSISTANT EXPERIMENTAL **OFFICER**

OR

### POSTGRADUATE STUDENT

Applicants should be graduates in Agriculture or other relevant subjects with practical agricultural experience. The person appointed will work under the supervision of Dr. M. J. Bryant on experiments concerned with body condition, long term nutrition and rebreeding of the sow during lactation. Three year project sponsored by the Agricultural Research Council. Further details and application forms, quoting ref: TN82, from Assistant Bursar (Per-sonnel), University of Reading, Whiteknights. Reading RG6 2AH. (1711)

### UNIVERSITY OF READING RESEARCH PROGRAMME: LEAF PROTEIN PRODUCTION

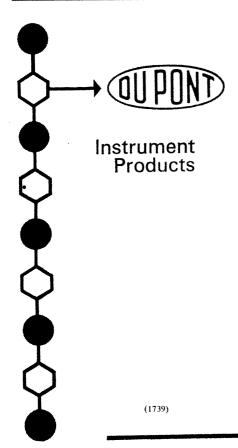
LEAF PROTEIN PRODUCTION

The Department of Agriculture and Horticulture has two vacancies in its programme for the Development of Optimal Agricultural Systems for Leaf Protein Production currently sponsored by the Wolfson Foundation. A RESEARCH OFFICER is required to install and maintain the large scale extraction equipment and to carry out the field scale cropping system trials. The successful applicant is likely to have a qualification in Agricultural Engineering with a strong practical interest in agriculture. Starting salary £1,600 to £2,500 p.a. according to qualifications and experience. A RESEARCH STUDENT is required to carry out factors affecting the accumulation of leaf protein in legume crops. Candidates should hold a First or Upper Second Degree in Agriculture, Agricultural Science or an appropriate Biological Science. The successful applicant will be required to register for a higher degree and the value of the Studentship will be £980 by £50 p.a. Both appointments are for three years and are to commence at the earliest date. Apply, as soon as possible, with the names of two referees to the Professor of Crop Production, Department of Agriculture and Horticulture, Earley Gate, Reading RG6 2AT, from whom further particulars can be obtained. (Ref. M. 51).

### UNIVERSITY OF ZAMBIA

UNIVERSITY OF ZAMBIA

Applications are invited for the post of (a) CHIEF or (b) SENIOR TECHNICIAN IN ANI-MAL SCIENCE, School of Agricultural Sciences. Applicants should possess a B.Sc. in Agriculture, or A.I.S.T., or A.I.M.L.T. or equivalent. Experience in Animal Nutrition and Biochemistry, Histology and/or Pathology, and Small Animal Management advantageous. Salary scales: (a) K3,300 to K4,900 p.a., (b) K2,900 to K3,500 p.a. (£1 sterling=K1.50). The British Government may supplement salaries for married appointees in range £456 to £516 p.a. (sterling) (normally free of all tax) and provide children's education allowances and holiday visit passages. This supplementation is unlikely to apply to single appointees. Family passages; various allowances; superannuation and medical aid schemes; regular overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, not later than November 27, 1974 to the Registrar, University of Zambia, P.O. Box 2379, Lusaka, Zambia. Applicants resident in U.K. should also send I copy to Inter-University Council, 1909 Tottenham Court Road, London WIP ODT. Further particulars may be obtained from either address. (1681)



# **Biochemist or Clinical Chemist**

# to be **aca Technical Specialist**

This wide-ranging role with the Du Pont Instrument Products Division results directly from the successful introduction of a new concept in clinical chemistry the Automatic Clinical Analyser. Introduced into Europe in 1973, after proven success in the USA, the ACA is capable of analysing a wide range of body fluids and reduces to a minimum the time between sample input and output of data.

After a comprehensive product training in the USA the person appointed will travel extensively in the UK, Scandinavia and the Netherlands, providing specialist support for unit sales, which are increasing. A detailed knowledge of clinical methodology gained through at least two years' laboratory experience is essential, and a knowledge of electronics would be useful. Previous experience in scientific/medical equipment would be helpful.

Starting salary will reflect the seniority of the appointment and benefits which are competitive with the best include a company car and relocation assistance where appropriate.

Please quote reference U.287 when writing or telephoning for an application form to:

T. T. Coulson, Personnel Services Co-ordinator, Du Pont (U.K.) Limited, Du Pont House, 18 Bream's Buildings, Fetter Lane, London EC4A 1HT. Tel: 01-242 9044, ext. 464.



#### STOWE SCHOOL, BUCKINGHAM

Laboratory Technician required to take charge of Biology Laboratory. Salary according to Buckinghamshire Educational Committee recommended scale (at present under revision) of £1,416 to £1,644 according to experience. The successful applicant will be eligible for housing allowance of £120 in addition, or alternatively a house or flats available on a service tenancy with a reduction of £110 from salary. New Laboratories have recently been opened.

Apply to the Senior Science Master, Stowe School, Buckingham, giving names and addresses of two referees. (1690)

#### UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG, SOUTH AFRICA SENIOR LECTURER IN PETROLOGY

PETROLOGY

Applications are invited for appointment to the above post in the Department of Geology. Duties are to be assumed on February 1, 1975.

Applicants should have substantial research experience in the field of igneous and/or metamorphic petrology, a familiarity with experimental petrologic techniques and a Ph.D. or equivalent qualifications. The salary presently attached to the post is in the range R7.245 to R9.315 and increased salary scales are to be introduced shortly. Benefits include an annual bonus, pension and medical aid facilities, and a housing subsidy, if eligible.

Intending applicants should obtain the information sheet relating to this post from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa, with whom applications should be lodged not later than November 30, 1974. U.K. applicants may obtain the information sheet from the London Representative, University of the Witwatersrand, 278 High Holborn, London, W.C.1, to whom a copy of the application should be sent. (1683)

### UNIVERSITY OF LONDON INSTITUTE OF NEUROLOGY

Postdoctoral Fellow to join a group investigating the biosynthesis of specific brain proteins during development. Biochemist or Cell Biologist preferred. Salary according to age and experience on the Lecturer scale starting at £2.118. London Allowance, F.S.S.U. Apply to Dr L. Lim, Department of Neurochemistry, Institute of Neurochemy N. 5.8 Exphange Court, London W.C. Department of Neurochemistry, Institu Neurology N, 5-8 Exchange Court, London OPP. Institute of ondon WC2v

### ROYAL POSTGRADUATE MEDICAL SCHOOL

#### JUNIOR TECHNICIAN/TECHNICIAN

required in Department of Clinical Cardiology. This post will involve dealing with patients and some research work. Qualifications GCE 'O' level passes in English, Maths and two Science subjects/ONC or equivalent. Salary according to qualifications and experience.

Applications to the Secretary PRMS Uservices

Applications to the Secretary, RPMS, Hammersmith Hospital, DuCane Road, London W. OHS, quoting ref. no. 2/354 N. (1704)

### UNIVERSITY OF PENNSYLVANIA

Applications are invited for a part-time Visiting Lecturer (one-year appointment) to teach Structural Geology (Spring Term) and Environmental Geology (Fall Term). It is anticipated that the position will be filled for the Spring Term starting mid-January 1975. Salary ranges from \$4,000 to \$6,000. Enquiries should be addressed to R. Ian Harker, Chairman, Department of Geology, D4, University of Pennsylvania, Philadelphia, Pennsylvania 19174, U.S.A. (1706)

### THE UNIVERSITY OF MARYLAND at College Park

Invites Applications for the Position of CHAIRMAN

of the Department of Physics and Astronomy

of the Department of Physics and Astronomy Applicants for the position should have administrative, research and teaching experience. The Department of Physics and Astronomy currently has 150 faculty members, 105 technical staff, and 64 non-technical staff. There are aver 200 undergraduate students and approximately 300 graduate students enrolled in programs leading to the B.S. M.S. and Ph.D. degrees. Nominations, applications and inquiries should be addressed to: P. F. Cumiff, Chairman of the Search Committee Office of the Division of Mathematical and Physical Sciences and Engineering, University of Maryland, Coffege Park, Maryland 20742.

The University is an equal opportunity/affirmative action employer.

### UNIVERSITY OF LONDON KING'S COLLEGE

DEPARTMENT OF ANATOMY

Applications are invited for the post of Lecturer or "Demonstrator" in the Department of Anatomy. There will be an obligation to engage in the teaching of Topographical anatomy Suitable candidates may be either seeking a career or preparing for the higher examinations in surgery. Salary scale £2.118 to £4.896 plus £213 London allowance. (Starting salary will be at an appropriate place on the scale.) F.S.S.U. benefit will be naid.

appropriate place on the Santa Santa

### **AUSTRALIA**

### **Public Service of Victoria Ministry for Conservation Fisheries and Wildlife Division**

# PROJECT CO-ORDINATOR

(FRESHWATER BIOLOGY), (REF. No. H/10) Yearly Salary \$A13,660

#### Duties:

To co-ordinate the participation of the freshwater fisheries staff in environmental studies. To be responsible for and participate in freshwater biological investigations in such studies and to maintain liaison with Government Departments, Universities and other groups conducting similar investigations. To prepare reports and recommendations.

### Qualifications:

An approved degree in Science or Agricultural Science or an equivalent qualification; preferably a higher degree. Ability to plan, lead and

co-ordinate activities involving multi-disciplinary research groups. Appropriate experience in freshwater biology and proven research ability. Experience in report preparation.

Applications quoting reference number (H/10), should be addressed to the Secretary, Public Service Board of Victoria, State Public Offices, No. 1 Treasury Place, Melbourne, 3002, Australia, by not later than 9.30 a.m. on Wednesday, November 20, 1974, together with statements of experience and qualifications and date and place of birth.

#### INSTITUTE OF CANCER RESEARCH LABORATORIES AT SUTTON, SURREY

LABORATORIES AT SUTTON, SURREY
Division of Tumour Immunology requires Biochemist-Research Assistant to work in Immunochemistry Laboratory, Experience in protein separation techniques desirable. Minimum qualifications, 1st Degree in Chemistry or Biochemistry, or 2 'A' levels in Science subjects. Registration for a higher degree can be considered. Post in Junior Technical Officer scale, salary £1,494 to £2,592 plus London Allowance (£126 p.a. currently under review) plus Threshold.

Apply in duplicate to the Secretary, Institute of Cancer Research, 34 Sumner Place SW7 3NU. giving the names of 2 referees, and quoting ref. 301/B/533.

(1708)

### M.R.C. CLINICAL RESEARCH CENTRE (NORTHWICK PARK HOSPITAL) WATFORD ROAD, HARROW, MIDDLESEX, HAI 3UJ **TECHNICIAN**

for work on the chemical study of some inborn errors of metabolism. The programme is particularly concerned with inherited metabolic diseases in which there appears to be a primary abnormality of organic acid metabolism and it would be suitable for a holder of H.N.C. in chemistry with experience in gas-chromatography. Ref. 123D/2B/4222.

#### **TECHNICIAN**

HECHNICIAN
with A.I.M.L.T. or H.N.C. preferably with experience of scrology, haematology or bacteriology to work in a laboratory concerned with the study of human immunological disease. Ref. 118/2/A21.
Salary for both posts within the range £1,986 to £2,961 plus Threshold increase.
Further details and application forms from Mrs J. Tucker-Bull.

Further details and app Tucker-Bull. Please quote reference.

### ROTHAMSTED EXPERIMENTAL **STATION**

### HARPENDEN, HERTS. AL5 2JQ ASSISTANT STATISTICIAN

to analyse and interpret data from agricultural and other biological experiments using the Station's computer. Degree, H.N.C. or equivalent qualification in mathematics or statistics. Some knowledge of agriculture or biology an advantage. The post is tenable until March 31, 1978 in the first instance and the person appointed will join a small team funded by the Overseas Development Administration to provide a statistical service for agricultural research workers in developing countries.

Appointment in grade of Scientific Officer

Appointment in grade of Scientific Officer (£1.592 to £2.675) plus threshold payments. Starting salary according to qualifications and experience. Superannuation with a contribution of 1½% for family benefits.

Apply in writing to the Secretary giving names and addresses of two referees and quoting reference 245 by November 29, 1974. (1731)

#### FACULTY POSITION

A developing physiology department is seeking individuals with active research programmes and publications in physiology and bio-physics. We are particularly interested in applicants with programmes in renal or endocrine physiology, but all outstanding applicants will be carefully considered. Teaching responsibilities include instruction at the graduate and undergraduate level. Reply to: Will J. Van der Kloot, Chairman, Physiology and Bio-Physics Department, Health Sciences Center, State University of New York at Stony Brook, Stony Brook, New York 11794 U.S.A. The Health Sciences Center is an Equal Opportunity Employer, and applications from members of minority groups and women are welcome. (1730)

### FIELD STUDIES COUNCIL DEPUTY WARDEN

at Juniper Hall Field Centre, near Dorking. Single, good degree in geography, educational qualifications. At least two years professional teaching experience including fieldwork. Wide environmental interests, but special knowledge of urban geography biogeography ad conservation an asset.

graphy biogeography ad conservation an asset.

Besides teaching, duties include administration (staff, student supervision, programme planning, equipment etc.) in consultation with Warden as his second in command. Research opportunities, Salary £1,800 by 75 to £2,025 plus free board and lodging. Appointment Winter 1974/75, closing date for applications November 18, 1974, Further details and application forms from The Director, Field Studies Council, Preston Montford, Montford Bridge, Shrewsbury SY4 1HW. (1735)

### UNIVERSITY OF MANCHESTER

DEPARTMENT OF PATHOLOGY

Applications are invited for a

### POST-DOCTORAL RESEARCH ASSISTANTSHIP

within a group working on the migration of lymphocytes and their role in immune responses. The post is supported by an M.R.C. programme grant to Professor W. L. Ford. The appointment is likely to be for three years.

Experience in some aspect of Cellular Immunology desirable. Salary range p.a.: £2,118 to £4,896. Enquiries may be addressed to Dr W. L. Ford, Dept. of Pathology, University of Edinburgh Medical School. Further particulars and application forms (returnable by November 21) from the Registrar, The University, Manchester. M13 9PL. Quote ref. 227/74/N. (1738)

### **AUSTRALIA**

### **Public Service of Victoria Ministry for Conservation**

# ENVIRONMENTAL MODELLER

REF. No. (H/12)

Yearly Salary \$A13,660

#### **Duties:**

To assess the role of mathematical modelling in environmental investigations and to be responsible for the implementation of modelling techniques. To assist in the co-ordination of environmental studies and in particular, of the interplay between environmental modelling and other aspects of environmental understanding. To maintain liaison with governmental agencies and other groups and to promote an understanding of the use of models as an aid to decision making.

### Qualifications:

An approved degree in the physical or biological sciences, or an approved degree in engineering or other qualification admitting to Membership in the Institution of Engineers,

Australia, or other appropriate qualification; a higher degree desirable. Considerable experience in environmental modelling and demonstrated ability in the application of modelling techniques to inter-disciplinary investigations. Exeperience in the practical application of modelling to conservation or environmental management an advantage.

Applications quoting reference number (H/12), should be addressed to the Secretary, Public Service Board of Victoria, State Public Offices, No. 1 Treasury Place, Melbourne, 3002, Australia, by not later than 9.30 a.m. on Wednesday, November 20, 1974, together with statements of experience and qualifications (1743)and date and place of birth.

### **BIOCHEMIST**

Pesticide Design Chesterford Park is the Research and Development centre for Fisons Agrochemical Division, which is responsible for the invention, development and manufacture of a wide range of chemical compounds for crop protection.

Transfer within the organisation means that we are now seeking an exceptionally able young scientist to contribute biochemically based ideas to our programme of rational pesticide design. The successful candidate will join an experienced team of synthesis chemists, and will also be responsible for supervising a range of biochemical assays to establish rational relationships between structure and activity.

Applicants, who would normally be within the age range 25-30, should be qualified to Ph.D lewel, ideally have some post-doctoral experience, and in addition, the demonstrated

ability to undertake innovative investigation at a high level. Previous experience of pesticide biochemistry is desirable rather than essential.

Starting salary will be commensurate with career record to date, the Company operates a first class pension scheme, and other conditions of employment are amongst the market leaders, Relocation assistance is available where appropriate,

For an application form, please write, quoting serial number 661/197 to:

R. J. Down, Personnel Officer, Fisons Limited, Agrochemical Division, Chesterford Park Research Station, Nr. Saffron Walden, Essex CB10 1XL.



(1746)

### **AUSTRALIA**

### Public Service of Victoria Ministry for Conservation

# ENVIRONMENTAL ECONOMIST

REF. No. (H/11)

Yearly Salary \$A13,660

#### **Duties:**

To be responsible for the development of the most effective means of applying economic theory and practice in environmental studies. To co-ordinate the activities of major environmental studies from an economic viewpoint and, in particular, to assist in the application of economics to environmental management. To prepare reports and to make recommendations.

### Qualifications:

An approved degree with economics as a major; preferably a higher degree. Proven ability to work effectively in multi-disciplinary

scientific investigations and wide experience in the application of economics to environmental investigations. Demonstrated ability at a senior level assisting in the planning and development of inter-disciplinary investigations.

Applications, quoting reference number (H/11), should be addressed to the Secretary, Public Service Board of Victoria, State Public Offices, No. 1 Treasury Place, Melbourne, 3002. Australia, by not later than 9.30 a.m. on Wednesday, November 20, 1974, together with statements of experience and qualifications and date and place of birth. (1744)

#### DIRECTOR OF THE PROPOSED CRUMP INSTITUTE OF MEDICAL ENGINEERING AT UCLA

Applications are invited for consideration by the Search Committee for the Director of the proposed "Crump Institute of Medical Engineering" at

Although the proposal for the formal establish-Although the proposal for the formal establishment of this unit is still under study by the Academic Senate and will require Presidential and Regental approval, the search for a highly qualified applicant has been initiated. This position represents a unique opportunity to contribute to the development of the evolving field of "medical engineering", and to establish a major Medical Engineering Programme at UCLA.

Obudifications: A person of asyestional ability.

Qualifications: A person of exceptional ability, accomplishments and international stature is sought whose work and interests bear on the interface between engineering and medical sciences. Demonstrated scientific productivity, leadership and administrative abilities are desirable.

Responsibilities: To administer and direct the proposed Institute.

Salary: Commensurate with qualifications and

Equal Opportunity: The University of California

is an equal opportunity employer.

Send resumé to Dr George Eisenman, Chairman, Search Committee, Department of Physiology, UCLA Medical School, Los Angeles, California (1713)

### UNIVERSITY OF NATAL DEPARTMENT OF PHYSICS

Applications are invited from suitably qualified persons for appointment to the post of

#### **LECTURER**

In addition to normal teaching duties, successful candidates will be expected to take part in the experimental or theoretical activities of one of the research groups of the Department. The fields of interest are:

- Magnetospheric and Atmospheric physics
- Laboratory plasma physics
- Electron Microscope studies of crystal defects

5. Electron Microscope studies of crystal defects 4. Fundamentals of quantum mechanics. Previous experience in one of these fields will be an advantage. The salary will be within the range: R5 520 to R7 935 per annum. A general improvement of salary scales is expected shortly. The commencing salary notch will be dependent on the qualifications and/or experience of the successful applicant. In addition, an annual vacation savings bonus is payable, subject to Treasury regulations.

regulations.

Application forms, further particulars of the post and information on pension, medical aid, staff bursary, housing loan and subsidy schemes, long leave conditions and travelling expenses on first appointment, are obtainable from the Registrar University of Natal, King George V Avenue, Durban, with whom applications, on the prescribed form, must be lodged not later than February 28, 1975, quoting Ref. Adv. 124/74. (1717)

### LIVERPOOL POLYTECHNIC 1. DEPARTMENT OF CHEMISTRY SENIOR LECTURER

in

### INDUSTRIAL CHEMISTRY/ CHEMICAL ENGINEERING

Applications are invited for the appointment of a Applications are invited for the appointment of a chemical engineer or chemist to join a team involved in the teaching and development of Industrial Chemistry to Honours degree level. The successful candidate will have a first or higher degree in chemical engineering or chemistry and the necessary experience of semi-technical scale plant to exploit fully the extensive facilities in the recently completed Industrial Chemistry Block. Research and consultancy will be encouraged. SALARY: £3,525 to £4,212 p.a. plus threshold

#### 2. SCHOOL OF PHARMACY

#### LECTURER II in PHARMACOLOGY

Applications are invited from graduates in Pharmacy or Pharmacology for the post of Lecturer II in Pharmacology. Applicants should hold a higher degree and relevant post-doctoral experience would be an advantage. The person appointed will be expected to teach pharmacology to honours degree students and to engage actively in research. SALARY: £2.200 to £3,474 p.a. plus threshold Details available from Staff Office, Dept. N. Liverpool Polytechnic, Richmond House, 1 Rumford Place, Liverpool, L3 9RH. (1719)

### UNIVERSITY OF TEXAS MARINE SCIENCE INSTITUTE

MARINE SCIENCE INSTITUTE

The University of Texas Marine Science Institute at Port Aransas is seeking applicants for an appointment, preferably at the ASSISTANT PROFESSOR level, from marine biologists capable of teaching courses in invertebrate zoology of marine organisms and of doing research in the area of physiological ecology. Applicants must have a strong research interest and a commitment to graduate training. Send curriculum vitae and a brief statement of research interests to the Director, The University of Texas Marine Science Institute, Port Aransas, Texas 78373. Affirmative Action/Equal Opportunity Employer. (1726)

### UNIVERSITY OF LIVERPOOL CHAIR OF PSYCHOLOGY

Applications are invited for the Chair of Psychology which will become vacant on September 30, 1975, following the retirement of Professor

30. 1975, following the retirement of Professor L. S. Hearnshaw. The salary will be within the range approved for full-time professional appointments and in any case not less than £6,264 per annum.

Applications, (12 copies), together with the names of three referees, should be received not later than December 6, 1974 by the undersigned, from whom further particulars may be obtained. (Candidates overseas may send one copy only by airmail). Quote ref. RV/293/N.

H. Burchnall.

H. H. Burchnall,

### **BIOCHEMIST**

required to work in clinical fields of abnormal proteins. Opportunities exist for thesis and training in clinical biochemistry.

Salary in accordance with Whitley Council A Scale according to qualifications and experience.

Please send applications with names of two referees to Professor J. R. Hobbs, Department of Chemical Pathology, Westminster Medical School, 17 Page Street, SWIP 2AR. (1733)

### CANCER RESEARCH CAMPAIGN RESEARCH TECHNICIAN (Chemist/Biochemist)

The Molecular Radiobiology group at the Campaign's Laboratory at Mount Vernon Hospital requires assistance with a wide-ranging research programme, investigating fundamental aspects of radiation damage in chemical and biochemical systems and using sophisticated physical techniques. We are seeking a person with proven experimental ability and experience in chemistry or biochemistry: formal academic background is less important. MRC scales apply, e.g. with HNC the post would probably be graded Technician (scale rising to £3,100 inclusive of London allowance and Threshold payments).

Send for further details and application form to: Executive Officer, CRC Gray Laboratory, Mount Vernon Hospital, Northwood, Middx HA6 2RN. (1747)

#### UNIVERSITY OF THE WITWATERSRAND **JOHANNESBURG**

NUCLEAR PHYSICS RESEARCH UNIT FIELD OFFICER:

GEOHYDROLOGIST

A vacancy exists in the Environmental Isotope Group for a suitably qualified person who is fond of an outdoor life and who will be required to work independently and exercise considerable initiative. Applicants should have at least a basic degree in Geology and should have some experience and/or further training in Geohydrology. Familiarity with the sinking and logging of boreholes as well as with geophysical techniques such as resistivity and self-potential will be useful.

Salary will be determined according to quali-

Salary will be determined according to qualifications and experience, and falls within the following ranges:

Senior Research Officer: R7245 to R9315 Research Officer: R5520 to R7935

Improved scales are to be introduced shortly.

The appointment will be for three years in the first instance, with the possibility of renewal.

Applications should reach the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, not later than November 30, 1974. A copy of the application should be sent to the London Representative, University of the Witwatersrand, 278 High Holborn, London WC1

# **Head of Theoretical Physics Group**

Applications are invited from theoretical physicists for the position of Head of the Theoretical Physics Group at Daresbury Laboratory, where members of the Group work in close collaboration with Universities using the Laboratory.

The Group is expected to play a key role in ensuring the effectiveness of the Laboratory's programme. This, at present, is in the fields of high energy physics, synchrotron radiation and computing, but a new major nuclear structure facility is under construction and the construction of a major purpose built source of X-ray and ultra violet synchrotron radiation is under urgent consideration by the Science Research Council.

The successful applicant will be expected to lead a team which will engage in research supporting the experimental programme at the Laboratory Salary will be assessed according to qualifications and experience, but is expected to be within the scale £6,700 to £7,750 per annum.

CLOSING DATE: November 30, 1974.

Please write or telephone Warrington 65000 Ext. 467 for an application form quoting reference DL/529/T to:—

(1727)



Personnel Officer Science Research Council Daresbury Laboratory, Daresbury, Warrington WA4 4AD

### FELLOWSHIPS AND **STUDENTSHIPS**

#### UNIVERSITY OF CALGARY DEPARTMENT OF CHEMISTRY BIOCHEMISTRY GROUP

Applications are invited for the position of **POSTDOCTORAL** RESEARCH FELLOW

commencing early in January 1975. Research will

commencing early in January 1975. Research will be concerned with the structure-function relationship of (1) proteolytic enzymes from Anthozoa; or (2) extracellular enzymes produced by thermophilic fungi.

The initial appointment would be for one year with provision for a second year. Salary according to NRC regulations (\$8,700 per annum).

Application with curriculum vitae and names of two referees to:

Dr K. J. Stevenson

Department of Chemistry
Biochemistry Group
University of Calgary
Calgary, Alberta, Canada
T2N 1N4. (1540)

(1540)

### THE ROYAL SOCIETY

Bruno Mendel Travelling Fellowships

Applications are invited for Bruno Mendel Travelling Fellowships up to a maximum of £5,000 per annum tenable for periods of up to 1 year from October 1, 1975 to enable postgraduate candidates to carry out experimental medical research in the United Kingdom. The Netherlands or Israel. Candidates should be normally resident in one of these three countries and should not already be living in the country which they propose to visit.

Application forms, receivable by January 15, 1975, and further details are available from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London, SWIY 5AG (1693)

### NEW ZEALAND

### UNIVERSITY OF CANTERBURY CHRISTCHURCH

### POSTDOCTORAL FELLOWSHIP IN CRYSTALLOGRAPHY

IN CRYSTALLOURATTI

Applications are invited for a one-year appointment to assist research aimed at relating the structural details of model compounds to the chemistry of oxygen transport in haemoglobin. The emolument will be NZ\$7,000 per annum. Further particulars, and Conditions of Appointment may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WCIH 0PF.

Applications close on November 30, 1974. (1694)

THE CITY UNIVERSITY Department of Chemistry

### POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a Postdoctoral Research Fellowship for work on the direct amination of hydrocarbons in collaboration with Dr R. G. Coombes and Mr B. O. Field. The appointment (Salary £2,118 plus Threshold Payments plus London Allowance with F.S.S.U. benefits) is tenable for one year from an early date to be arranged by agreement and may be renewable.

Applicants should be good experimentalists with backgrounds in either organic or inorganic chemistry or have experience of heterogeneous catalysis.

Applications giving a curriculum vitae and naming two referees, as

soon as possible, please to:
Dr R. G. Coombes,
Department of Chemistry,
The City University,
St John Street, London ECIV 4PB. (1737)

### TWO POSTDOCTORAL FELLOWSHIPS IN THE EARTH AND PLANETARY SCIENCES

The Lamont-Doherty Geological Observatory of Columbia University invites scientists interested in any field of the earth and planetary sciences to apply for the following Fellow-

Two Post-Doctoral Fellowships, each awarded for a peroid of one year (extendable to two years in special instances) beginning in September, 1975, with a stipend of \$12,000 per annum. Completed applications are to be returned by February 15. Application forms may be obtained by writing to the Director, Lamont-Doherty Geological Observatory, Palisades, New York, 10964.

Award announcements will be made March 31, 1975.

(1652)

### THE UNIVERSITY OF HULL

DEPARTMENT OF PLANT BIOLOGY Applications are invited for the post of

### POSTDOCTORAL FELLOW IN PLANT BIOCHEMISTRY

IN PLANT BIOCHEMISTRY
to work with Dr. D. R. Threlfall on the nature
and properties of the enzymes involved in the
biosynthesis of vitamin E and related compounds
by higher plants and micro-organisms.
The post is financed by the S.R.C. and is
tenable until October 31, 1975.
Salary (excluding threshold payments) will be
on the scale £2,226 by £114 to £2,340 by £72 to
£2,412, plus F.S.S.U. benefits.
Applications giving details of age, qualifications
and experience together with the names of two
referees should be sent to Dr. D. R. Threlfall,
Department of Plant Biology, The University
Hull HU6 7RX, from whom further particulars
may be obtained.

### UNIVERSITY OF LEICESTER

DEPARTMENT OF ENGINEERING Applications are invited for a post of either

### RESEARCH FELLOW or EXPERIMENTAL OFFICER

on an SRC-supported two-year research project into high current-density electrochemical machining with molten salt electrolytes.

Applicants should have either a higher degree or at least two years postgraduate experience, or a first degree, respectively: corresponding salary in the range £2,118 to £2,757 or £1,650 to £2,178 plus threshold.

Further details and application forms may be

of the range £2,118 to £2,777 of £1,630 to £2,170 plus threshold.

Further details and application forms may be obtained from the Head of the Engineering Department, The University, Leicester LE1 7RH (Ref. RF3). Informal enquiries to Mr. H. E. Freer or Dr. A. C. Baxter. (1696)

### POSTDOCTORAL FELLOW OR RESEARCH ASSOCIATE

Available immediately for research into laboratory culture and studies of larval blackflies in conjunction with the program on biocontrol. Salary commensurate with experience and qualifications. Send application etc. to Dr. M. Laird, Director, Research Unit on Vector Pathology, Memorial University of Newfoundland, St. John's, Newfoundland. (1673)

#### UNIVERSITY OF BATH Applications are invited for RESEARCH STUDENTSHIP

supported by S.R.C. for research into plant steroids, as part of an existing programme, Gradu-ates in pharmacy, botany and biochemistry should

Please write with full details to Dr. R. Hardman School of Pharmacy & Pharmacology, The University, Bath, BA2 7AY. (1675)

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SCHOOL OF CHEMISTRY

### RESEARCH STUDENTSHIP

Applications are invited for appointment to a research studentship in the field of Corrosion. The appointment will initially be for two years renewable for a third.

The work will involve the study of various inhibitors in their protective action on steel in corrosion media, in collaboration with Atlas Products and Services Limited.

The candidate should hold a 1st or 2nd Class Degree in Chemistry or Metallurgy or an equivalent qualification. The successful applicant will be encouraged to register for the degree of M.Phil. or Ph.D. (C.N.A.A.).

Salary: £1,544 by £55(2) to £1,654 pius payments under the threshold agreement

Further particulars and form of application may be obtained from the Secretary, Thanses Polytechnic, Wellington Street, London SE18 6PF, to whom completed applications should be returned by November 5, 1974. (1697)

### GILLETTE INTERNATIONAL RESEARCH FELLOWSHIP IN PHYSICS

UNIVERSITY OF SUSSEX, ENGLAND APPLICATIONS ARE INVITED FOR AN INTERNATIONAL POST-DOCTORAL RESEARCH FELLOWSHIP

set up by the Gillette Company to promote the international exchange of scientific and technical workers. The present Fellowship is tenable in the School of Mathematical and Physical Sciences of the University of Sussex under the direction of Professor M. W. Thompson.

Applicants should hold a higher degree, or have had 3-4 years post-graduate research experience. This Fellowship is open to nationals of the U.K., other Western European countries or the United States, or Canada.

The Fellow will be expected to undertake studies of the interaction of accelerated ion beams with solids or the generation of ion beams for materials technology. Applicants may submit their own proposals for a programme of work, although the University cannot guarantee to accept them. It is expected that the results of the work will be published.

The Fellowship will be for one year, in the first instance, with the possibility of renewal for a further year and will carry a remuneration of about £2,800 per annum. Applicants should be able to take up the Fellowship during 1975.

Applications giving full personal particulars including curriculum vitae and the names and addresses of two academic or technical referees, should be sent to:

The Secretary,
International Research Fellowship Committee.

International Research Fellov Gillette Research Laboratory. 454 Basingstoke Road, Reading, Berkshire, England.

Closing date for applications January 31, 1975

(1725)

### UNIVERSITY OF LEEDS DEPARTMENT OF PHYSICAL CHEMISTRY

Applications are invited for the post of RESEARCH FELLOW

work with Professor P. Gray and Dr. A. Clifford.

to work with Professor P. Gray and Dr. A. A. Clifford.

The person appointed will work on the measurement of the diffusion rates of hydrogen atoms in gases, using the technique of resonance fluorescence. Applicants should hold a Ph.D. degree and have experience of research in a related field. Salary on the scale £2,118 to £2,412.

Appointment will be for one year in the first instance, with possible renewal for a further year. Applications to Dr. A. A. Clifford, Department of Physical Chemistry, the University, Leeds LS2 9JT, by November 30.

#### CITY OF LONDON POLYTECHNIC SIR JOHN CASS SCHOOL OF SCIENCE AND TECHNOLOGY

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(2) An S.R.C. C.A.S.E. Research Studentship for study of colloidal clay particles.

Applicants should have a good 1st degree, M.Sc or M.Phil in a relevant science and will be required to register for a higher degree.

Applications forms and further details from the Secretary of the Physics Department, City of London Polytechnic, Jewry Street, London ECS1 2EY (Tel No. 01-283 1030 Ext. 309). (1670)

### POLYTECHNIC OF THE SOUTH BANK DEPARTMENT OF PHYSICS POSTDOCTORAL RESEARCH **FELLOW**

Applications for the above post are invited from suitably qualified candidates to take part in a programme of work based on the application of nuclear techniques, including the use of fast neutron and charged particle beams, to problems in proteins and the suitable problems. in materials analysis

in materials analysis.

Further details of the work currently being carried out in the department and of the conditions of appointment may be obtained from:

Mr. A. J. L. Collinson,
Head of Department of Physics,
Polytechnic of the South Bank,
Borough Road, London SEI 0AA.
Tel: 01-928 8989 Ext. 2160.

(1671)

### THE HEBREW UNIVERSITY OF **JERUSALEM**

#### THE FACULTY OF SCIENCE

Applications are invited for Postdoctoral Fellow-ships for the academic year 1975-1976 In the Fields of Mathematics, Physics, Chemistry, Life & Earth Sciences

The applicant is required to submit his applica-tion (in letter form) before December 1, 1974, together with his list of publications and curriculum vitae, containing details of his marital status, to the Chairman of the Institute, in which he is interested to carry out his research. Applications for post-doctoral research in Earth Sciences should be submitted to the appropriate Head of Department, i.e., Geology, Geography or Atmospheric Sciences. All applicants are requested to address copies of their correspondence to the Dean, Faculty of Science. Science.

The applicant is further required to arrange for

The applicant is further required to arrange for at least two letters of recommendation, from persons well-acquainted with his personal and academic record, to be directed to the relevant Chairman, or Head of Department: these recommendations to reach their destination by December 1, 1974.

The fellowship provides for a tax-free salary ranging from approximately IL.14,000 to IL. 16,000 (dependent on the candidate's marital status), which is payable in eleven monthly instalments. The faculty usually allows for a single air-ticket to Israel, and a similar allowance is made if the candidate leaves Israel on completion of his tenure as post-doctoral fellow.

The fellowship is intended primarily for persons

his tenure as post-doctoral renow.

The fellowship is intended primarily for persons who attained their degree in very recent years, and for those who will complete all requirements for the Ph.D. degree before the autumn of 1975.

(1651)

### UNIVERSITY OF KENT AT CANTERBURY RESEARCH FELLOWSHIP IN PHYSICS

Applications are invited for a post-doctoral Research Fellowship supported by the Science Research Council to join a group using laser light scattering techniques for the study of molecular motions in simple liquids and solids. Experience in high resolution optical spectroscopy or computerised data analysis would be an advantage but is not essential. Salary in the range £2.118 to £2.412. Application forms and particulars may be obtained from the Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent CT2 7NH, to whom completed applications should be returned by November 13. Please quote ref. A72/74.

### POSTDOCTORAL FELLOWSHIPS

Subject to approval of funds postdoctoral opportunities are anticipated in the following areas of biting fly research program on biocontrol; 1. Ecology of larval blackflies; 2. Laboratory studies and culture of pathogens and parasites of biting flies, and 3. Field surveys for biting fly pathegens and parasites. Send application etc. to Dr M. Laird, Director, Research Unit on Vector Pathology, Memorial University of Newfoundland, St. John's, Newfoundland. (1707)

### SHEFFIELD POLYTECHNIC RESEARCH FELLOW IN BIOLOGICAL SCIENCES

Applicants should be experienced in microbial biochemistry, with particular reference to biological recycling of industrial and town wastes and effluents.

The initial appointment will be for three years with the possibility of extension for a further two years.

Salary Scale: Senior Lecturer Grade £3,525 to £3,945 (bar) to £4,212 Plus Threshold

Application forms and further details are obtainable from the Personnel Officer, Sheffield Polytechnic, Halfords House, Fitzalan Square, Sheffield S1 2BB, to whom completed forms should be returned as soon as nossible (1718)

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Further information from:
The Conference Secretariat:
Dr. Nicole Müller-Bérat,
Statens Seruminstitut,
Amager Boulevard 80
Copenhagen S,
Denmark,

or the I.S.D. Secretariat: Dr. Dimitri Viza, Laboratoire d'Immunobiologie, Faculté de Médecine Pitié-Salpétrière, 105 boulevard de l'Hôpital, 75634 Paris Cedex 13, France.

Deadline for submitting abstracts: 15th May, 1975.

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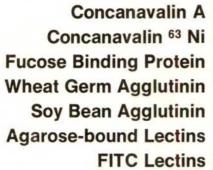
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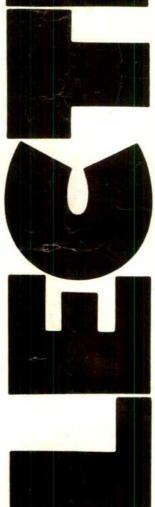
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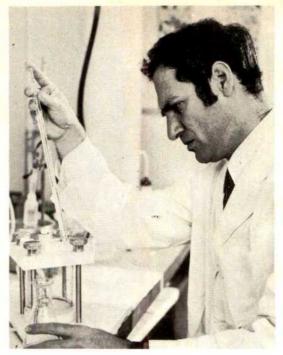
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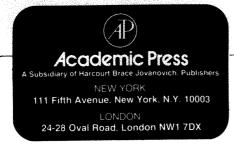
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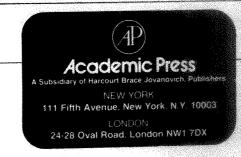
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### **Cover Picture**

Aldous Huxley-a 1935 portrait by Cecil Beaton. William Cooper reviews a biography of Huxley on p. 129,



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Volume 252

November 8, 1974

### **Support for Trieste**

THE International Centre for Theoretical Physics in Trieste was established in the early sixties in order to provide a base to which young competent theoretical physicists from developing countries could go occasionally for intellectual exchanges and advanced research. An extensive report on the centre's activities was given earlier this year in Nature of March 22. Much of the early, and indeed continuing success has been due to the manipulations of Abdus Salam, then Pakistan's delegate to the International Atomic Energy Agency (IAEA) and Paolo Budini, professor of physics at the University of Trieste. These two managed to persuade IAEA, the city of Trieste and the Rome government to support a project which encountered a lot of cynicism and disbelief from the scientifically advanced countries. Many felt that helping physicists from technologically backward countries to pursue esoteric research was wasteful of scarce manpower resources. Salam vigorously attacks this point of view by pointing to the alternative open to bright young physicists who discover the exhilaration of research emigration. In a developing country there may be a mere handful of highly skilled academic physicists', he declares, 'They each play a major role in the development of that country's technology, universities and intellectual life. Better far to provide somewhere to recharge their batteries than simply to let them emigrate.'

In the early days the city provided a total of \$1 million and IAEA gave \$55 thousand annually. UNESCO started with \$27 thousand annually and in 1970 moved up to a fuller level of participation. At present the Italian government gives \$350 thousand, UNESCO, the UN Development Project and IAEA each give \$200 thousand.

UNESCO support, although modest at first, is, at least formally, ten years old and UNESCO pursues a policy of regarding its financial aid as no more than seed money to get an institution going. After ten years, according to its protocol, the centre should be able to stand on its own feet and find sources of money elsewhere. This is a sound policy for most establishments that UNESCO supports which can go to a specific national government and ask for money. But it is less sound where an institute is used by 90 different nations, the majority poor.

The official British line, which will lead to the delegation expressing their concern at UNESCO this week, is that after ten years there are other deserving projects which could use the \$200 thousand—itself a large slice of the total UNESCO basic science budget of \$1.5 million—and so the support should be re-examined. And a 34% growth rate, they say, is out of line with other growth rates in UNESCO. There is no immediate pressure on Trieste, as it is not proposed to cut funding at once; however in the long run it is clear that the British delegation want Trieste to look elsewhere for their money. Other countries do not see it that way, indeed

one or two diplomat-scientists privately express the view that Britain is making herself look foolish by such a gesture at this time. With inflation posing terrible problems for all educational institutions and the rest of the world apparently unready to rock the boat, what causes Britain to take a mayerick line?

Partly, no doubt, there is a feeling in the Ministry of Overseas Development (which concerns itself with UNESCO) that the way to help developing countries is through more 'relevant' research. But the prime mover in this affair seems to have been a distinguished scientist himself—Sir Harold Thompson, chairman of the Royal Society UNESCO scientific committee.

It had been clear for some time that Sir Harold intended to urge UNESCO to withdraw from Trieste. His views on the centre (*Nature*, 243, 136; 1973) were that it was successful, but he did express some concern that international laboratories were being backed by 'well organised pressure groups'. Yet it was not until May 1974 that a group of physicists and mathematicians familiar with the centre was convened by the Royal Society to put their point of view to Sir Harold.

The meeting seems to have been unsatisfactory in that there was no real meeting of minds over the issue. The group, pressure group that it doubtless was, left dissatisfied and with the feeling that the nature of the Trieste centre and the problems that it faces had been barely understood. Trieste's mathematical activities—a recent development—had hardly been appreciated.

As a result, Britain's official viewpoint cannot really be said to be representative of the views of British physicists and mathematicians and seems as much as anything to have emerged from one man's opinion.

The growth rate of 34% quoted by those who urge re-examination is a mystery. It is a real growth rate, we were assured. And yet UNESCO's contribution has risen only from \$150 thousand to \$200 thousand so far in the seventies, and only inflation is permitted to modify this figure before 1976. Hardly a real growth of 34% and certainly not per annum.

It is, of course, entirely within Sir Harold's rights to press for a strict interpretation of the UNESCO rules if he wishes. But surely when so many others are deeply concerned, an opinion which flies in the face of the advice of fellow scientists is worrying, and a mechanism by which a Royal Society-appointed committee can go to an international meeting with an un-representative view needs examination. And the figures should be right.



A LARGE monumental fountain, ornamented by the celebrated sculptor Carpeaux, has been erected on the Observatoire Place at Paris. It represents Europe, Asia, Africa, and America rotating the globe, which they carry on their heads, and is very effective; but in spite of M. Le Verrier's protestations, they are rotating the globe from east to west, according to the Ptolemean theory.

# international news

# Monod's answer to the Pasteur's problems

THE financial problems of the Pasteur Institute have long been clear to the French public and to workers at this famous French biological research institute. The Pasteur has been in the red for the past 15 years and has been obliged to conduct many fund-raising campaigns through the press and television, as well as seeking government aid.

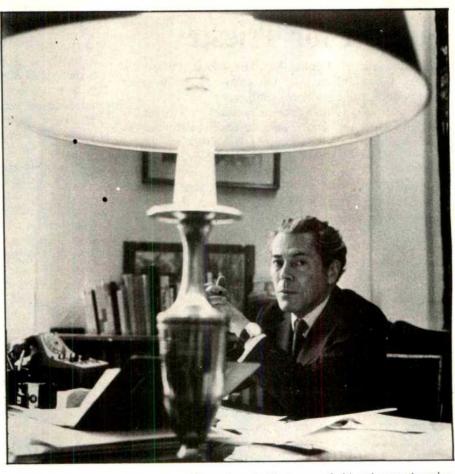
It now seems that this has not been enough to save the situation, which has been further aggravated recently in an unforeseeable way.

At a meeting on October 11, Professor Jacques Monod, director of the institute, announced to the scientific staff that there was a risk that employees and suppliers would not be paid after May 1975. Most of the assets of the Pasteur Institute have already been sold and no more credit can be obtained.

The annual amount spent by the institute will reach about 65 million francs by the end of 1974, of which various government contracts will pay for around a third. Unfortunately the institute cannot count on profits from its new factory for producing vaccines and sera, set up in Normandy in 1973. Although sales are satisfactory, production costs have risen much higher than expected. Profits are thus much lower than predicted and financial equilibrium is several years off.

Added to this gloomy financial situation is the deplorable condition of many of the buildings centred around the Rue Doctor Roux. Only the departments of molecular biology and virology occupy new or renovated buildings. The department of bacteriology is scattered over several buildings, some of which date from the time of Louis Pasteur himself. In some cases even normal security precautions cannot be enforced. In crumbling lecture theatres, students have to write on their knees. The building of a long awaited new immunology department seems to have been adjourned sine die for financial and administrative reasons.

Unless a great deal of work is started quickly, Professor Monod fears that a creeping paralysis will overcome work



Monod at the Pasteur; a bold and attractive plan.

at the institute. But in the present parlous state of the finances, even the urgent improvements cannot be started.

To disentangle the institute from this mess, Professor Monod has just presented a bold and attractive plan to the institute's Council of Administration and the public authorities. He wishes to sell all the buildings and land in the Rue Doctor Roux and rebuild the institute at an entirely new site at Garches, 10 kilometres to the southwest of Paris. Because of the enormous rise in land values in the centre of Paris, the sale would raise sufficient funds to enable the institute to build its new laboratories and still leave a profit of 70 million francs. An operation on this scale could, of course, not be realised without government approval. Also, many of the scientists, although admiring Professor Monod's effort and imagination, ask whether the project is realistic in the present uncertain economic climate in France. The fact that the financial forecasts for past years have turned out to be wide of the mark

and that details of the financial situation remain unknown to most of the workers, clearly raise doubts among the researchers about the validity of the new project. The Pasteur Institute is being asked if all possible efforts have been made to find a less grandiose but less risky solution, and it is feared that the financial and technical crisis may precipitate a crisis amongst the staff.

Whatever the final solution turns out to be, it will be a painful one for the institute and those who work there. Research teams may not survive the upheavals of the next few years and some researchers and technicians may well leave the institute.

At the end of November, the government representatives on the Administrative Council will give the government's decision and a final decision will be arrived at on December 19. In the meantime, M. Royer, the President of the Administrative Councils plans to hold a meeting with the staff in the absence of Professor Monod.

FROM THE STAFF OF LA RECHERCHE.

Soviet government to the United States from straightforward dissidence, "caus- himself. Nevertheless, the gravity of about the position of Jewish would-be ing the hospital to harbour incorrect the charges is evoking considerable emigrants seems to promise a consider- ideological tendencies" and "accepting concern in the West, and a number of able improvement in the lot of those bribes from patients", to attempted eminent scientists and doctors, includscientists dismissed from their posts murder of child patients. This last ing Lord Hunt of Fawley, Professor and subsequently subjected to official accusation, on which Dr Shtern cur- William Stanley Peart, and Professor harrassment as a consequence of their rently faces trial, represents a new Sir Ludwig Guttman have taken part application for a visa for Israel. That trend in the pattern of accusation— in a campaign of letters, telegrams and is, provided a serious attempt is made would be emigrants having been in-telephone calls to the All-Union and to implement them! A spokesman for the Medical and Scientific Committee for Soviet Jewry admitted, however, to a "certain amount of scepticism' concerning the announcements. "When we actually see the bargain being implemented . . . , when the activists in Moscow are allowed to go, when dicted so far mainly on relatively minor Voronel is free from harassment and charges of "hooliganism". To the this seminar allowed to function with- observer, it seems reminiscent, on the out interruption, then we shall feel that one hand, of the notorious 'Doctors' our work has borne fruit. Not until plot" of the last days of Stalinism and, then. Meanwhile Voronel is once on the other, of an atavistic remore in hiding and the trial of Dr emergence of the old "ritual murder" Shtern is due to begin."

The case of Dr Mikhail Shtern, Director of the Department of Endo- senious than any so far brought, the crinology at a hospital in Vinnitsa pattern is running true to form. It refusal of the Vinnitsa Procurator's (Ukrainian SSR) does, indeed, seem to hardly needs the statements made last Office to reveal any details of the projustify this cautious attitude. Dr Shtern, September by two relatives of former ceedings may be a first stage in the and his wife applied in September 1973 patients of Dr Shtern, that the investi- tacit dropping of the charges as part for visas for Israel. Since then they gating authorities have been compel- of the new agreement. Even if this have been subjected to continuous and ling patients to give false testimony should be the case, the manner of the systematic harassment, including sear- that "the doctor whom they had deed, the almost sadistic detailing of ches of their apartment, the dismissal trusted and respected for 30 years in- the prisoner's medical condition to reof their sons, both scientists, and their tended and attempted to poison their latives allowed no access to him, can sons' wives, from their professional children", to see in this prosecution hardly be seen as a favourable augury posts, and the interrogation of Dr the usual harassment of the would-be for the promised new deal for Soviet Shtern himself on a number of charges, emigrant; indeed, in May, the Pro-Jews.

THE recent assurances given by the These have changed, over the months, curator of Vinnitsa admitted as much

### Promises, promises

from Vera Rich, London

charge.

Although the charge is far more

Ukrainian Medical Academies, and the Vinnitsa Procurator's Office-so far without any effective reply.

The trial was scheduled for October 30 but enquiries made by Dr Shtern's sons, Viktor and Avgust, to the Procurator's office, elicited only the information that the legal proceedings were "no business" of theirs. The only news forthcoming is that the 56-yearold doctor is in a serious state of health, with internal haemorrhage from a duodenal ulcer and tuberculosis. He is at present being held in an underground room and is to be visited by a medical commission.

It is, of course, possible that the

WITH talk of massive cutbacks in federal spending to help fight inflation, and with the 1976 budget just three months from publication, some members of the scientific community in the United States are getting the jitters. And well they might, for expenditures on science and technology fall almost totally in the so-called controllable category of the federal budget, thus making them prime targets for reduction.

One area which is clearly going to be hard pressed is high energy physics, and the Zero Gradient Synchrotron (ZGS), a proton accelerator located near Chicago, is a likely candidate for extinction. The Office of Management and Budget (OMB), which prepares the Administration's budget request, has already decided that the ZGS should soon be consigned to the technological scrap heap, for earlier this year it asked the Atomic Energy Commission and the National Science Foundation to develop a "plan for shutting down the ZGS accelerator at the earliest possible time".

The rationale for that decision is that since money for high energy physics is unlikely to grow in these

### Foot on the brake for accelerators

by Colin Norman, Washington

times of financial stringency, and with the new Fermi National Accelerator soaking up a rapidly growing proportion of funds available for that esoteric science there just is not enough money to operate all the particle accelerators at a reasonable level. The ZGS machine being the least powerful of the high energy accelerators, and also being located close to the new Fermi accelerator, looked like the logical one to shut down.

There is, of course, nothing new in such a trend. To accommodate the growing operating budget for the Fermi machine-which began work about two years ago-the Atomic Energy Commission cut off funds for the Princeton-Pennsylvania accelerator in 1971, axed the Cambridge Electron Accelerator a year later, and is now in the process of converting the Bevatron from a high energy machine into a heavy ion accelerator.

But, having decided that the ZGS should be the next to go, the office of Management and Budget must have been astonished at the advice it received on how the deed should be done. A special AEC-NSF committee which was set up to develop a plan for shutting down the ZGS, told the budget cutters in an unpublished report last month that it found whe ZGS programme to be "vigorous and innovative" and that it could see "no scientific or technical reason to recommend a shutdown at this time". Ask a silly question

The committee pointed out that some features of the ZGS are unique, and that just because it is operated at a relatively low energy does not necessarily mean that it is the most expendable of the high energy accelerators. To confuse the OMB people even further, the committee recommended that operation of the ZGS should be continued "possibly at a more intensive level" for the next four years, and that mid-1979 should be considered the earliest reasonable time to shut the machine down.

### Radical medicine in Beersheba

from Nechemia Meyers

ISRAEL'S fourth medical school, attached to the Ben-Gurion University of the Negev, opened this month in Beersheba with a first-year class of 34 students, two of them Arabs.

Was there any justification for yet another medical school in a country with the world's highest ratio of physicians to potential patients (about three per thousand) and hundreds of students at existing schools, not to speak of the 1,500 Israelis studying medicine in Europe? Many people, particularly those connected with the other three medical schools, think not. But thanks to the missionary zeal of its chief advocate, Professor Moshe Prywes, it is now an established fact.

Prywes, connected for many years with the Hebrew University Medical School in Jerusalem, is determined to develop a very different kind of institution at the Ben-Gurion University, of which he is president.

He wants to use the new school as a means of breaking down the barriers that now exist between academic medical centres on one hand, and neighbourhood clinics on the other. At present, Israel's best qualified doctors, graduates of her own medical schools, cluster around the universities hospitals, university-affiliated leaving day-to-day medical care to be dispensed mainly by old-timers and new immigrants. And the neighbourhood doctors, being effectively cut off from hospitals and research centres, tend to practice out-of-date, conveyorbelt medicine-which makes them frustrated and their patients dissatisfied.

Prwyes aims to ensure that the graduates of his school are different by training them to be patient-oriented from the outset. For example, the schools calls for students to work in clinics and hospitals, under the supervision of special tutors, from their very first year.

Internship will also be handled in a new way. Instead of becoming interns at the end of their studies, the fledgling medicos will spend a full year working in the field, albeit with limited responsibilities, midway through their course.

The Ben-Gurion University Medical School, unlike others in Israel, will play down basic biological research, emphasising instead research linked to the specific health problems of the community. While not in any way denying its importance, the directors of the new school advise would-be students who are primarily interested in research to enrol elsewhere.

This same philosophy is reflected in

the selection of members of faculty, among whom are men who have never written a scientific paper in their lives. In Prywes's opinion, physicians who have proved themselves as lecturers and as doctors in the field are as qualified to be professors as are research-oriented MDs with dozens of papers to their credit.

These unorthodox professors, it is hoped, will set an example for their students. "Hitherto," Prywes has pointed out, "neither the medical student nor the resident saw his teachers working outside the hospital or the research laboratory. Thus talking to students about the importance of community medicine was bound to be a waste of time. They could hardly be convinced of the significance of something their own mentors ignored."

### Stormy weather hits Mexican research

FLOODS and very early frosts have destroyed several years' worth of research at the International Wheat and Maize Improvement Center, the internationally renowned plant breeding institute run by Norman E. Borlaug in Mexico. Frost killed off most of the hybrid wheat and maize being bred at two research stations near Mexico City early in September, and floods associated with hurricane Fifi completely devasted a third station in eastern Mexico three weeks later.

In most cases the crops—which represented the results of two or three years of cross breeding-were destroved before they had produced seed for planting next year. According to Dr Robert Osler, deputy director of the center, there is no reserve seed from intermediate hybrids so it will be necessary to go right back to the original strains and cross breed them again over the next two or three years to reproduce the destroyed plants.

Osler said that up to 80% of the wheat and the entire maize crop was destroyed at a station at Toluca near Mexico City by frosts which arrived several weeks earlier than usual. The same frosts also wiped out most of the maize at another station on the other side of Mexico City, although much of the wheat crop there survived. Osler fears, however, that because the frost caught the plants at a relatively early stage the seeds may be difficult to germinate.

Flood damage to the third station is more serious. Torrential rain associated with hurricane Fifi-the one which devastated Honduras-caused a river to burst its banks and flood fields at a research station located at Poza Rica to a depth of 3 metres. Osler said that the water took the topsoil off a

large area and flooded silos and machinery sheds.

Osler estimated that it could take at least a year to repair the damage, one problem being that replacing the topsoil could introduce a good deal of variability in soil condition. The Center has applied to the Consultative Group, an international group of donors which supports crop breeding research, for funds to repair the damage. There is also a possibility that a European government may provide at least part of the money.

### Rhodesia rules

"THE University [of Rhodesia] has been exempted by the British Government and others from sanctions." So runs the last sentence of a sheet describing Salisbury, its university and the chair of biochemistry which was being advertised recently. What does this mean in practice? One of our readers enquired of the Foreign Office in London and got the following replies:

Can I convert European currency into Rhodesian currency? Travellers to Rhodesia are normally only permitted to take £25 in sterling notes.

What personal goods can I take? Household and personal effects, but not

Can I order and receive scientific research equipment? Export to Rhodesia is prohibited except by licence issued only for humanitarian reasons (religious, educational or medical). Each application is considered separately; present policy is to give "sympathetic consideration to applications from the University of Rhodesia"

Can I advertise in the United Kingdom for technical and administrative personnel? Only under licence (the same criteria apply as above). Present policy is to issue licences for academic but not for other posts.

Can I seek research grants in the United Kingdom? Nothing prevents you, but the transfer of funds is subject to strict exchange control ruleseach case is considered on its merits.

Can I change Rhodesian currency back into other currencies? It cannot be exchanged in the United Kingdom. "I cannot say what facilities are available in Rhodesia.'

He's not going.

We called the Foreign Office for clarification of some items and they draw attention to two points. First, nothing can be taken for granted in dealing with the United Kingdom from Rhodesia. There are no automatic exemptions and everything has to be reviewed. Second, there is no British representative in Rhodesia, so none of the customary consular facilities either of a routine or an emergency nature are available.

THE abrupt departure last week of Dr tioned its appearance on the Washing- bill to dissolve the AEC and replace it John Sawhill as head of the Federal ton landscape. Energy Administration (FEA) came as survive, and although he was generally ordinating committee which consisted regarded as an effective administrator who won praise from Congress and consumer organisations, he has trodden on a good many important toes recently.

His resignation—which can be described as forced rather than voluntary -paved the way for the appointment of Andrew E. Gibson as the new administrator of the FEA. Gibson's background is not in energy policy but in maritime affairs—he is a former Maritime Administrator and Assistant Secretary of Commerce—but that is not surprising since relevant experience has rarely been a criterion for appoint- of top officials of government agencies programme. It is that factor which has ment to a top energy job in either the carrying out energy programmes. Nixon or Ford Administrations.

it is instructive to examine some of the an Energy Resources Council as the bureaucratic musical chairs which have overall manager of the federal govern- appointed to fill a newly created post been taking place in energy policy since ment's energy policies and named his in the State Department. The result of April last year. At that time John old Congressional friend Rogers C. B. a reorganisation plan dictated by Con-Ehrlichman was nominally in charge Morton, the Secretary of the Interior, gress, the position is designed to elevate of energy planning in his capacity as as its chairman. That put Morton firmly scientific affairs in the department by Chairman of the Domestic Council, in charge (he also retains his job as bringing them together. but when he became otherwise engaged Secretary of the Interior) and higher in Nixon created a National Energy Office the energy bureaucracy than Sawhill • Two reports published recently by to advise on policy.

That arrangement lasted only a few White House to coordinate energy programmes. He persuaded John Love forced his resignation. to resign as Governor of Colorado to consultant.

Love's reign lasted only until October, when Nixon announced yet another • On the same day that Sawhill's as head of the office and Love, em- of the Atomic Energy Commission NSF suggests. bittered by his shoddy treatment, re- (AEC), has been appointed Assistant turned to Colorado where he is now Secretary of State for Oceans and for only the second time in 20 years, practising law. DiBona also departed to International Environmental and Scien- the number of scientists and engineers become executive director of the petro- tific Affairs, and William A. Anders, a employed by the government dropped leum industry's chief lobbying office. former astronaut, has been named as last year. In October 1972, there were The Federal Energy Office's name was head of the new Nuclear Regulatory 166,700 scientists and engineers on the later changed to the Federal Energy Commission (NRC). The shuffle is the federal payroll, but a year later the

In April this year, Simon became no surprise to those who have been Secretary of the Treasury following the will take charge of virtually all of the keeping track of the jockeying for departure of George Shultz from the federal government's energy research power and political backstabbing government, and Sawhill, who was which has characterised energy plan-Simon's deputy, was promoted to head ning in the United States for the past the agency. But it is important to note 18 months. Sawhill, after all, had been that Simon retained a lever on energy in the job for six months, which is planning because he hung on to the longer than anybody else managed to chairmanship of an inter-agency co-



### Washington seen

by Colin Norman

To put last week's events in context, Ford, however, who last month created and Simon. Since the two are said to be the

In view of that chronicle of events, the Administration.

Administration when Congress sanc- result of Congressional approval of a number had declined to 161,500.

with the ERDA and the NRC

Seamans, an aeronautical engineer, and development programme, and his appointment has so far been generally welcomed. He faces a tough job, however, in getting the ERDA off the ground since bureaucratic jealousies are sure to arise when programmes are shifted from existing federal agencies into the new organisation. And, since the ERDA will be built around the laboratories of the Atomic Energy Commission, he will be faced with the difficult task of striking a balance between nuclear and non-nuclear research.

The appointment of Anders has, however, drawn mixed reaction. As head of the NRC, Anders will be placed in charge of regulating nuclear power plants and ensuring that they meet safety criteria. He has been a Commissioner of the AEC for some 15 months, where his chief responsibility has been to oversee the breeder reactor drawn some sniping, since the breeder All that was changed by President reactor has become one of the chief targets of nuclear critics

Finally, Dixy Lee Ray has been

National Science Foundation at loggerheads on some issues and since (NSF) catalogue a trend of declining weeks before Nixon scrapped it and Sawhill recently annoyed White House federal budgets for science and techformed an Energy Policy Office in the officials by openly advocating that a nology and of declining employment of 30% tax be slapped on gasoline, Morton scientists and engineers by the federal government.

First, in spite of modest increases in come to Washington as the new energy it is small wonder that a coherent spending on science and technology in czar and DiBona was retained as a energy policy has yet to emerge from the past two years, the NSF reports that when inflation is taken into account, the science budget for this year is "below that of any year during the reorganisation which entailed scrapping resignation was announced, another 1965-75 decade." In short, expenditures the Energy Policy Office and creating a shuffle of top officials concerned with on research and development have Federal Energy Office, a more powerful scientific matters took place in Wash- climbed from \$16,800 million in 1973 body with a large staff and a mandate ington. Robert Seamans, President of to \$17,700 million in the financial year to develop energy policies and to carry the National Academy of Engineering 1973-74 and \$19,600 million this year. out day-to-day operations. William and former Secretary of the Air Force, But, in terms of 1967 dollars, the pur-Simon, a spectacularly successful Wall was named as head of the new Energy chasing power of the science budget Street investor who was then Deputy Research and Development Administra- has declined from about \$17,500 million Secretary of the Treasury, was named tion (ERDA), Dixy Lee Ray, Chairman in 1967 to about \$13,000 million, the

Another set of statistics shows that,

# India parcels out its energy problems

from Narender Sehgal

RECENT changes in the central council of ministers, following a cabinet reshuffle, have resulted in the creation of a separate Ministry for Energy, to be headed by a minister of state (slightly lower in status than a full fledged cabinet minister). This, however, does not mean that all subjects relating to energy have been brought under one roof. Far from it; as of now, only 'power' (from the erstwhile Irrigation and Power Ministry) and 'coal' (from 'mines' which until now had been under the charge of the Ministry of Steel and Mines) have been assigned to the new ministry.

Fears are already being voiced about the administrative problems that may arise from the change. For example, much of the hydroelectric power produced (37% of the total generated in India by all means) is tied to irrigation projects, hence their association in a single ministry. (Irrigation will now form part of the Ministry of Agriculture.)

Oil, which accounts for 50% of India's entire consumption of energy derived from commercial sources, remains, as before, part of Petroleum and Chemicals under the charge of a cabinet minister.

The diversity of coal users (railways and the steel and fertiliser industries, for example) presents an additional problem of administration and coordination. It remains to be seen if it is possible to retain all matters concerning coal within the ambit of the newly created Ministry of Energy.

Then there is atomic energy which remains under the direct control of the Prime Minister. In this area of increasing importance, Shrimati Gandhi has been in the past assisted by Shri K. C. Pant who will continue in this role even after he has taken charge of the new Ministry of Energy.

On the basis of the past record of co-operation and coordination between various ministries, it is not possible to feel overly enthusiastic about this new attempt to solve India's grave and growing energy problems.

• India is known to have uranium in only small amounts. Known deposits, at present rates of production and consumption (with due allowance for planned future uses) would last no longer than some 30 years. On the other hand, China is understood to possess uranium in fair abundance in relation to her immediate and long term needs. One would think, then, that China could not possibly have any use for Indian uranium.

No so. Men arrested by the Bihar police recently, with varying quantities of uranium in their possession, have revealed that the stolen metal was meant to be smuggled to China through Nepal and that another 100 kilograms or so was awaiting disposal at various places in Bihar. An editor of the Hindi daily at Patna, engaged in investigating the smuggling operations and possessing some related documents, was reportedly murdered and relevant documents stolen by the apparently well organised gang of uranium smugglers, just before the newspaper was about to publish the story.

The uranium in question was stolen from the Jadugoda mines near Jamshedpur in Bihar. While investigations and more arrests are continuing, the view is gaining ground that the motivation behind the Chinese interest in Indian uranium may have to do with her desire to weaken or slow India's atomic energy programme.

## Weather watch down under

by John Gribbin

THE Commonwealth Meteorology Research Centre, which was set up in 1969 with a five year lease of life, has recently been reconstituted and provided with an extension for a further five years, under the auspices of the Australian government. Now the Australian Numerical Meteorology Research Centre (ANMRC), this Melbourne based operation is playing a key role in studies of the problems of meteorological research in the southern hemisphere.

These problems are formidable indeed. Because of the great proportion of ocean in the south the observational network is far from complete; in addition, the distribution of what land there is means that population in the southern hemisphere is concentrated in the tropical and near tropical regions, north of 40°S. And far less is known about the behaviour of weather systems at these latitudes than at extratropical latitudes.

During its first five years of existence under the "Commonwealth" label, the centre was closely allied with the Global Atmospheric Research Programme (GARP), and this spirit seems likely to continue. In July of this year, when the centre was renamed, the Australian government affirmed support for the next five years, with research objectives defined in the following terms:

"The work of the Centre consists of studies of the behaviour of the earth's atmosphere, with emphasis on general circulation, directed towards improvement in the accuracy and timescale of weather forecasting, and towards improvement in understanding the distribution and variations in climate on the earth."

The work at the ANMRC is, as its name suggests, strongly orientated towards computer studies, with facilities available on the IBM 360/65 at the World Meteorological Centre in Melbourne (this centre is the southern counterpart of the two WMCs in Washington and Moscow, the three forming part of the World Weather Watch). With the aid of modern satellite photography, many of the handicaps resulting from the shortage of observing stations in the south can be overcome; satellite and other data are combined in mathematical models of atmospheric flow which have already produced reasonable forecasts for one to four days ahead. Improvements are likely to come on two fronts: through introducing more representations of physical processes into the model (better 'skill'); and through refining the numerical methods used (better 'efficiency').

In global terms, the Australian work is particularly interesting because it offers an alternative mathematical approach to some of the problems which beset meteorologists attempting to produce global models. So far, such models have encountered difficulty because of problems near the poles, and although progress is being made there is a need for different approaches to resolve the difficulties. The ANMRC has been working with the so-called 'spectral method', which involves representing the atmospheric variables by a truncated series of spherical harmonics, and with a combination of spectral techniques and grid techniques, which they refer to as a 'semi-spectral' or Fourier model.

In a draft version of part of the 1974 Annual Report of the ANMRC (as yet unpublished) it is stated that "a significant proportion of the operational products [sic] now issued by the National Meteorological Analysis Centre of the Australian Bureau of Meteorology are numerically derived", although "manual intervention" is still an important part of the forecasting process. The draft goes on to say that the Fourier model is now also proving useful in basic atmospheric research, and is helping to provide an insight into the dynamics of the stratosphere and mesosphere; in future years, as the research programme matures, the ANMRC sees a greater emphasis on such problems as the influence of volcanic debris and of anomalies in sea temperatures on climatic surface 

### The language of space

from Angela Croome

Lt.-Colonel Alexeit Leonov, commander designate of the Soyuz crew for next summer's Apollo-Soyuz linkup (the ASTP) announced a nice compromise on the question of space communications during the joint presentation on ASTP progress at the Amsterdam International Astronautical Congress. Last year it was not known what the language of space would be, said Leonov; it had now been decided that on the mission the Russians would speak English and the Americans Russian. In an emergency each team would revert to its own language. It had not however been fixed who would take overall command in a crisis.

The 'book' of operational proce-

dures had been written and practised in the four joint training sessions. Emergency procedures had yet to be worked out and there was further polishing of the language to be done; this had proved much more of a problem than originally envisaged (though judging from Leonov's competent performance, his English was no longer a headache). Still to be fully sorted out was the ticklish question of the interrelation of the two control centres. There seemed no clear indication that an American team of controllers would be sitting in at Baikonur next July

Both the Russians and the Americans assured questioners that there was nothing to choose between the two sides in terms of input to the engineering and other design and operational concepts. Nonetheless behind the

scenes American space planners were saying the flow of information was all one way and in the space medicine field in particular there was a great scarcity of adequate Soviet data. How had the Soviet programme eradicated early problems with motion sickness? Skylab astronauts, groggy and sick during their first week in the orbital workshop, would dearly like to know.

### Medallurgy

We are agog to see whether our hint (Nature, 246, 113; 1973) that the Royal Society's policy for the distribution of medals was just a trifle inward-looking has fallen on fertile ground. It's medalstime again next week and several Fellows have told us they will try and persuade Council to look further afield this year.

# correspondence

### Radioactivity salted away

SIR-Referring to the article "Fruits of a Faustian bargain" (Nature, 251, 274; 1974) it appears that the United States propose tests in 1980 for the storing of radioactive waste so as to stop dumping it—as do the UK, USSR and other nations—in the ocean. The German authorities seem to be ahead of them, as for several years now, low and moderately radioactive waste has been stored in a salt mine close to Hannover by the Gesellschaft für Strahlen-und Umweltforschung owned by the Federal German Republic. And there is still space for thousands of barrels for decades to come. It may be taken for granted that no earthquake will occur to wreak havoc-for millions of years none has happened there. so the only thing to fear is mankind itself by trying to prove its efficiency by discovering a new and final way of self destruction.

HANS K. KOENBER

Laser & Electro-optik, D-8000 München

### Plasmid moratorium

SIR—The appeal by a committee of eminent biomedical scientists for a voluntary moratorium on an area of scientific research which may create unpredictable hazards to human health (Nature, 250, 175; 1974) reminded me of a talk which Leo Szilard gave at a writers' club in Moscow in December 1960 which I attended, and where to my knowledge the question of a moratorium was raised for the first time. This story was later told by Szilard

and published1.

"A year ago last December I was in Moscow to attend the sixth Pugwash conference. And while I was there I was invited to talk to a writers' club about molecular biology, about my work. One of these writers said, 'Now what practical consequences does this have?' So I said, 'As far as I can see it has no practical utility whatever—but of course if you had asked me that about nuclear physics in the 1930s I would have told you the same thing.' And then the Russian said, 'Well in that case, wouldn't it be better if you stopped right now?'".

Yours faithfully, Gertrud Weiss Szilard

La Jolla, California 92037 <sup>1</sup> Thinking Ahead with Leo Szilard, in Int. Sci. Tech., 33–38 (1962).

### Sitting Bull?

SIR-I was startled to read (Nature, 251, xviii; 1974) that Colorado State University is encouraging 'ethnic minorities and women" to apply for the position of Chairperson in its Statistics Department. I reject as highly improbable the implicit assumption that ethnic minorities necessarily consist entirely of males. Nevertheless, I wonder whether the aim is to encourage collective applications resulting in large-scale migrations of American Indians (that is deviations from their normal distribution). Does this mean that sophisticated statistical tests (excluding Student's t, of course) will then be used to select individual chairpeople

from large samples? And, even if this analysis is at variance with the real object of the exercise, won't the unsuccessful applicants sioux?

Yours faithfully.

F. A. SMITH

Department of Botany, University of Adelaide, Adelaide, South Australia 5001

### ILLL reactor?

SIR,—Although apposite and allusive alliterations are always amusing and appropriate as acronyms, Institut Laue-Langevin still satisfies many of us. It recalls that the construction of this long awaited reactor is owed to a Franco-German agreement, whilst with 1,000 visitors and 300 completed experiments in the last year, the Institut is already known as ILL the world over.

I believe we should 'let ILL alone'.
Yours faithfully,

W. M. LOMER

Institut Laue-Langevin, Grenoble, France

### Erratum

SIR In my news note (Nature, August 9) credit for the fallopian tube transplant experiments in animals should have gone to Dr B. M. Cohen of the University of Cape Town Medical School, not to his close colleague Dr M. Katz, who has drawn my attention to the error. I apologise to both gentlemen.

IAN RIDPATH

35 Oakwood Gardens, Ilford, Essex

# news and views

# How to have your fibrous cake and eat it

FIBRE reinforced plastic composites combine the high strength and modulus of strong fibres with a high work of fracture (toughness). Two letters in this issue describe methods of improving the work of fracture without significantly reducing the strength.

A high work of fracture allows one to use a material secure in the knowledge that in a structure it will show signs of failure long before it actually breaks. It might be thought that the combination of brittle fibres with a brittle resin would produce a brittle composite. The toughness of these materials is the result of energy-absorbing processes at the weak fibre-resin interface during fracture. The most important of these processes is probably the work of pulling out broken fibres after the crack tip has passed, leaving a whiskery fracture surface. This work decreases as the interfacial bond strength is increased because the crack then cuts more cleanly through the fibres leaving shorter pull-outs. On the other hand, to weaken the bond strength also weakens the composite.

A new approach demonstrated by Atkins (page 116) is to produce alternating strong and weak bonded regions on the fibres. The strong zones maintain the composite strength, the weak regions allow lengths of fibres to debond, break and pull out so toughening the material. He uses boron fibre-epoxy resin composites with bands of silicone grease or polyurethane varnish on the fibres to produce weak bonding while, he claims, the uncoated regions will be strongly bonded. The varnish-coated composite shows a remarkable increase in toughness at coating levels above 50% and the strength is maintained up to 90% coating. For the silicone grease the toughness increase is much more modest. The results all fit Atkins's analyses for strength and toughness. The increase in toughness with varnish coating

is from 50 kJ m $^{-2}$  with no coating to 200 kJ m $^{-2}$  at 90% coating with no strength loss. Conventional surface treatments would only be expected to produce variations of a few per cent about the lower value without also changing the strength.

What is not clear yet is whether the varnish and grease both do act as weak coatings relative to uncoated fibres. The difference in the results for the two coatings suggests that the full explanation is more complicated. The system is obviously of commercial interest but also could be scientifically productive since our knowledge of this interface (like that of most interfaces between different phases or materials) is very limited. Carbon fibre reinforced plastics may be expected to show similar improvements but it is interesting to note that the silane treatments used on glass fibres are known often to form beads on the glass surface rather than smooth coatings.

A second approach to toughening is described by Gordon and Jeronimidis (page 116), who have considered the arrangement of cellulose fibrillae in the cell walls of timber. Gordon has remarked before that trees seem to have opted for a high work of fracture rather than maximum strength or modulus. Reasoning that this might be associated with the helical arrangement of the fibrils around the cell wall, Gordon and Jeronimidis now show that under stress the helical tube collapses and the structure undergoes extensive deformation before breaking. They report that model composites constructed on the same basis show very high work of fracture values (400 kJ m<sup>-2</sup>). They report no strength or modulus measurements but theoretically the decrease in modulus should be small and presumably strength will behave similarly.

Many other approaches to improving the low toughness of boron and carbon fibre reinforced composites are also being studied. But there is a long way to go before materials of the complexity of bone, ligament or timber are developed and before the material is designed concurrently with the structure in which it will be used.

P. D. CALVERT

### Micrometeoroid density from lunar craters

THE Solar System not only contains planets, moons, asteroids, comets, meteorites and meteorids but also a plethora of smaller particles known as micrometeoroids. These are so small that they do not burn out when they enter the Earth's atmosphere but are gently retarded by momentum transfer to the air molecules and slowly float to the ground. All particles with masses less than 10-6g fall into this category, the Earth's surface collecting 3,000 tonnes of this material each year, enough to form a layer of dust 1 cm deep during the Earth's lifetime. The physical properties of these particles can only be measured indirectly. Experiments flown on satellites and space probes measure mass, velocity and sometimes orbit. Rockets can be used to collect the particles in the atmosphere but only pick up the micrometeoroids after they have been retarded by the atmosphere and contaminated by meteoric dust ablated from larger meteoroids. Studies of zodiacal light lead to values for the particles' dielectric constant and shape.

A new and exciting way of studying the properties of micrometeoroids is by investigating the surfaces of the small glassy spherules found in the samples of lunar soil returned to Earth by the Apollo and Luna space programmes. These spherules are formed like raindrops during the solidification of the molten ejecta blown out from the lunar surface during impact cratering and volcanic events. They then lie on the surface, exposed to space and to the micrometeoroid influx. As the Moon has no retarding atmosphere the micrometeoroids strike the spherules at velocities between 5 and 20 km s<sup>-1</sup> and produce small craters.

Spherules have around 5,000 microcraters (diameters a few  $\mu$ m) per square centimetre of their exposed surface, the numbers of craters increasing with decreasing crater diameter. A typical microcrater is shown in Fig. 1.

Smith, Adams and Khan have concluded—in a communication on page 101 of this issue of *Nature*—that by comparing the ratio of crater depth to diameter in microcraters on the surface of lunar spherules with this ratio for laboratory-produced impact craters in glasses they can estimate the densities of micrometeoroids, the percent-

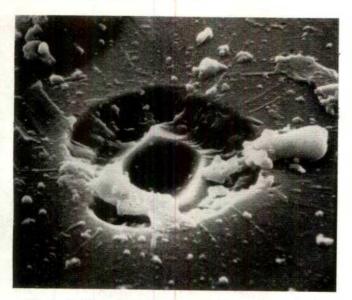


Fig. 1 A 13 µm impact crater on a lunar glass spherule. The spall zone has removed the raised rim and contains radial and concentric fracture patterns (NASA S-71-45362: courtesy of Friedrich Hörz).

ages of the micrometeoroid influx having differing densities and the way this percentage varies with particle mass. They find that the microcraters fall into three distinct groups having depth to diameter ratios of 0.94 (deep craters), 0.50 and 0.33 (shallow craters). By measuring craters produced by impacting hypervelocity (~3 km s<sup>-1</sup>) iron, aluminium and polystyrene pellets on to soda lime glass, tektite glass and quartz the authors conclude that the ratio of crater depth to diameter decreases when the projectile density decreases and that the three different ratio values found for lunar microcraters are produced by iron micrometeoroids (density 7.9 g cm<sup>-3</sup>) stones and carbonaceous chondrite micrometeoroids (density 2.7 g cm-3) and low density (1.2 g cm<sup>-3</sup>) particles, possibly spongy micrometeoroids or even ice crystals. The perentages of high, medium and low density particles in the  $\sim 2 \mu m$  diameter range are 40, 20 and 40% respectively. Measurements were also made in the laboratory of the ratio between the crater diameter and the diameter of the incident particle. The authors find that iron particles produce craters of twice their own diameter, the spongy micrometeoroids producing craters having the same diameter as the projectile-so the mass of the projectile responsible for a crater can now be calculated easily. Considering particles with diameters greater than 2 µm the incident mass is found to be made up of 20% iron micrometeoroids, 10% stony micrometeoroids and 70% spongy micrometeoroids. So spongy micrometeoroids which for a long time have been thought to be the predominant type in the radio meteor range of the mass spectrum (that is, >10<sup>-6</sup> g), also predominate down to about 10-10 g, a result which indicates that some differentiation process must have occurred during the production of these small particles.

Many events have been suggested to explain the origin of micrometeoroids—among them being debris from asteroidal collisions, condensations in the outer solar corona, accretion and condensation of interstellar material, remnants of the primordial dust cloud that produced the planets and debris from decaying comets. This new result should help to narrow the choice. It can be compared with observations of meteorites, the large bodies (>10° g) which penetrate the atmosphere and can be subsequently collected and analysed. Here irons make up 6% by number, stones and stony irons 94%. So these two types of particle of very different size have entirely different relative proportions of irons and stones.

Smith, Adams and Khan also find that the nature of

micrometeoritic material depends strongly on the region of the mass spectrum being considered, the proportion of iron particles increasing as particle size decreases. The region with particle diameters between 1 and 2 µm (mass ~4×10<sup>-12</sup>g) is found to be a region of transition, larger particles having predominantly low densities, smaller particles having predominantly high densities. This is another very important result because it could be the first definite observation of the effect of radiation pressure on the micrometeoroid dust cloud. In the Solar System the force due to radiation pressure opposes that due to solar gravitational attraction and the two forces cancel out for spherical particles at 1 AU when the product of the particle diameter and density equals 1.2×10-4 g cm-2. For particles of densities of 7 and 1 g cm<sup>-3</sup> the limiting diameters are 0.2 and 1 µm respectively, smaller particles being quickly blown out of the solar system. 1 µm should then be the 'cut off' size for low density particles and this is exactly what Smith, Adams and Khan have found. For smaller particles to have stable orbits they must have high densities. Assuming that 7.9 g cm<sup>-3</sup> is the highest particle density an extrapolation of the authors' results indicates that no particles of mass less than 10-14 g can come within 1 AU of the Sun and have a stable orbit. So the increase in particle numbers with decreasing mass should halt at 10-14 g. Smaller particles than this should be very rare, the few present having been produced in the recent past and moving in hyperbolic orbits as they escape from the Solar System. But this prediction is difficult to check because 10-14 g is right at the limit of detection for satellite, space probe and lunar cratering experiments.

DAVID W. HUGHES

# The linear differentiation of chromosomes

From the first description of an induced differential staining of plant chromosomes following cold treatment (Darlington and La Cour, J. Genet., 40, 185; 1940) twenty-eight years were to elapse before Caspersson and his colleagues (Expl Cell Res., 49, 219; 1968) showed that chromosomes treated with quinacrine mustard displayed a series of lateral fluorescent bands. This technique became known as Q banding and was quickly followed in other laboratories by the development of the C, G, R, G<sub>II</sub>, T, N and Cd banding and the DNA/RNA hybridisation in situ techniques. These techniques have had great value in the recognition of individual chromosomes and the analysis of translocations and other rearrangements-since extended to all hybrids, especially man-rodent hybrids. They have introduced to human cytogenetics refinements comparable with the contribution of the salivary gland chromosomes to Drosophila cytogenetics. In addition, they have stimulated speculation about the biophysical and biochemical organisation of chromosomes which this article attempts to review.

Discovery of the causes of C, G and Q banding should illuminate the long-standing distinction between constitutive heterochromatin and euchromatin, now found to be repetitive and unique DNA sequences respectively. Most methods involve treatment of acetic/alcohol fixed chromosome spreads in solutions of varying pH, temperature, and ionic strength followed by staining with Giemsa or fluorescent dyes, though the age and quality of preparation are found to be of great importance. The principles are often more important than the actual recipe and the successful outcome is so variable that the methods have been compared to Chinese cooking (Mammalian Chromosome News Letter, 15, No. 2; 1974).

Histone distribution, AT/GC base ratio and denaturation

### Maintaining genetic diversity

Among the many recommendations of the United Nations Conference on the Human Environment (Stockholm, 1972) which are beginning to bear fruit are those on the conservation of genetic resources. Registration of bacterial culture collections and seed banks is already under way. Now an *Index of Living Plant Collections in the British Isles* has been compiled by Dr J. T. Williams (Bentham-Moxon Trust, Royal Botanic Gardens, Kew, 1974). In his introduction to the index Professor Heslop-Harrison says that "the compilation of the present index was not inspired directly by the UN recommendations [having been started before the Stockholm conference] but its intent is wholly consonant with them".

This preliminary index lists institutions with collections of higher plants and collections actively being used in research. It is being distributed worldwide by the International Union for Conservation of Nature and Natural Resources; possibly also by the Food and Agriculture Organisation of the United Nations, as a step towards the ultimate aim of an international register of living plant collections.

of DNA were each proposed as possible causes of Q, G and C banding patterns. Histones however were shown to be partially extracted by fixation and even when they were completely removed by HCl post-treatment G banding was not affected (Comings and Avelino, Expl Cell Res., 86, 202; 1974). Similarly, we have observed (Ockey, Nobel Symp., 23, 344; 1972) that removal of histone labelled with tritiated precursors is uncorrelated either negatively or positively with G or C banding. Since proteolytic enzymes alone induce G banding and fixation does not seriously denature DNA, differential denaturation was also ruled out as the main cause of Q and G banding. But C banding requires alkali treatment which does induce denaturation with a subsequent rapid renaturation of the highly repetitive DNA sequences in the SSC stage of treatment. Other factors are however involved. Comings and his colleagues (Expl Cell. Res., 77, 469; 1973) and Pathak and Arrighi (Cytogenet. Cell Genet., 12, 414; 1973) both showed that DNA is lost preferentially from the non-C band regions; this may therefore explain their subsequent Giemsa differentiation but by quantitative autoradiography we have observed (unpublished) that when older <sup>3</sup>H-thymidine labelled preparations are used C bands are visible in many chromosomes that show equal loss of DNA from C and non-C band regions.

The C-banding technique is related to the earlier technique of DNA/RNA hybridisation in situ (Pardue and Gall, Science, 168, 1356; 1970) using RNA transcribed from satellite DNA. Both involve the denaturation of DNA and both are supposed to locate constitutive heterochromatin yet there may still be some discrepancies between them. The human Y chromosome has always shown up as constitutive heterochromatin in C banding but until now had given no indication of repetitive DNA by the hybridisation method. Recently, however, Evans and his

colleagues (Nature, 251, 346; 1974) showed that the Y contained repetitive sequences of all the four AT-rich satellites so far fractionated from human DNA. Constitutive heterochromatin was originally regarded as being ATrich, presumably because satellite DNA was AT-rich but this has been found not to be the rule. Other results have indicated that constitutive heterochromatin regions may react differently to different techniques. Some C-banding sites are non-fluorescent with quinacrine as in the human karyotype, but the Y chromosome, which also contains C band repetitive sequences, is the most fluorescent chromosome of the complement. Hoechst 33258, a more recent fluorescent dye, stains the ATrich C bands in the mouse but not O bands while in Microtus both typical O and C bands are stained (Hilwig and Gropp, Expl Cell Res., 75, 122; 1972). Facultative heterochromatin (that of the inactive human X which is represented by the late replicating sexchromatin body in interphase) behaves like the active X with all known banding techniques, whereas most of the other forms of late replicating heterochromatin can be differentiated by Q and G banding.

It has been proposed that because guanine quenches fluorescence, Q bands must be AT-rich, but this has proved a false trail. Recent work, both with synthetic DNA polymers and with AT-rich satellite DNAs would suggest that the important considerations for quinacrine fluorescence were the sequential arrangement of base pairs, particularly the periodicity of guanine residues which show localised quenching of AT-rich regions, and the availability of binding sites (Selander and de la Chapelle, Nature new Biol., 245, 240; 1973; Weisblum, Nature, 246, 150; 1973). The recent synthetic polymer studies with Hoechst 33258 by Weisblum and Haenssler (Chromosoma, 46, 255; 1974) show that fluorescence of this compound is enhanced by both AT- and GC-rich DNA, enhancement being greater however with AT-rich DNA.

Gottesfeld and his colleagues (Biochemistry, 13, 2937; 1974) have carried these studies a stage further with detailed biophysical study separated euchromatin and heterochromatin, and found that quinacrine fluorescence was quenched more with euchromatin than with heterochromatin. The DNA extracted from these fractions however showed similar AT/ GC base ratios and a similar fluorescence. They suggest from circular dichroism spectroscopy studies that heterochromatin DNA probably exists in the B configuration while euchromatin shows the C form. It is interesting to note in this connection that the conformation of AT-rich DNA in bacteria varies very much in its configuration with relative humidity and over a narrow range of ionic strength of NaCl (Bram and Tougard, Nature new Biol., 239, 128; 1972), both of which are critical factors in banding.

The incorporation of base analogues into DNA also affects the staining behaviour of chromosomes. Zakharov and his colleagues (Chromosoma, 44, 343; 1974) have found a considerable reduction in chromosome spiralisation at metaphase in regions which incorporate bromodeoxyuridine (BrdU) and BrdC during the latter period of the S phase when heterochromatin is replicating. differential spiralisation No such was observed when the analogue was incorporated into the early replicating euchromatin, implying a difference in conformation of the DNA between the two forms of chromatin as suggested by Gottesfeld and his group.

Latt (Science, 185, 74; 1974) showed that BrdU, incorporated semiconservatively over two cell cycles, resulted in one of the chromatids showing strong fluorescence (monofilial incorporation), the other (bifilial incorporation) weak fluorescence using the dye Hoechst 33258. Latt explained this difference as a quenching in the bifilial chromatid with less or no quenching in the monofilial BrdU chromatid. This technique provides a valuable tool in analysis of sister-chromatid exchanges (Kato, Nature, 251, 70; 1974). Perry and Wolff (Nature, 251, 156; 1974) were able to obtain a permanent differential staining of chromatids following SSC treatment after BrdU incorporation and subsequent Giemsa staining. The bifilial chromatid was less intensely stained and less condensed that its sister. These results they suggest are probably due to a difference in protein binding to BrdU-containing DNA. They also obtained differential fluorescence with acridine orange in the two chromatids.

Bobrow and Gross (Nature, 251, 77;

1974) have found that in mousehuman hybrid cells the contrasting  $G_{11}$ banding behaviour of the parental chromosomes is retained in the hybrid. This will prove not only a valuable asset in karyotype analysis of hybrid cells but also indicates that whatever characteristic of the DNA is responsible for its Gu banding response persists even in a hybrid nucleoplasm. Others have approached this problem by studying the structure of the Giemsa molecule involved in staining. Sumner and Evans (Expl Cell Res., 81, 236; 1973) suggest that the structures of the Giemsa and quinacrine molecules are such that they are able to bridge longitudinally separated sites on the chromosome which have been brought into close proximity by folding of the DNA, and that the spatial arrangement of these sites is influenced by the nonhistone proteins. The various banding techniques would then affect the cohesion or the biochemical nature of the non-histone binding. Studies using stains whose chemical specificity is known indicate the possibility of a differential distribution of -SH and -SS- groups in the bands (Sumner, Expl Cell Res., 83, 438; 1974) or that free NH2 groups are the binding sites for banding with fluorescent dyes like dansyl chloride (Utakoji and Matsukuma, Expl. Cell. Res., 87, 111; 1974).

R, G<sub>11</sub>, T, N and C<sub>4</sub> banding may all prove equally relevant to an analysis of the biochemistry and biophysics of chromosomes, but their contribution to date is at a much earlier stage. Progress in understanding the longer established techniques has been slow, partly because few biochemists worked with the cytologists who had made the great technical advances. The many current attempts to explain banding reflect our lack of knowledge of how I cm of DNA can be packed with protein into a 1 mm transverse band. But the challenge has now been accepted by the biochemists and the results should soon be fruitful.

> C. H. OCKEY A. J. BATEMAN

# Retiring Crab and gamma-ray bursts

from Andrew Fabian and James Pringle
An informal symposium held at the Institute of Astronomy, Cambridge on October 18 brought together observers and theorists to discuss two separate topics—the Crab Nebula and cosmic γ-ray bursts. Renewed interest is being shown in the Crab Nebula, the remnant of the 1054 AD supernova, because a series of occultations of the nebula by the Moon has just begun. These give experimenters a further opportunity to measure its size and structure. Ten

years ago the Naval Research Laboratory group observed the rate at which the 1-10 keV X-ray flux decreased as the Moon covered the nebula and were thus able to deduce a size of 110±25 arc minutes. Now it should be possible to obtain more accurate results, and at a variety of wavelengths.

Professor Walter Lewin (Massachusetts Institute of Technology) gave a report of his August 13 balloon observations made in northern Canada. The observation was made in the 20-50 keV range and, thanks to the accuracy of the occultation prediction, both the disappearance and reappearance of the nebula were observed. The initial indications are that the emitting region is asymmetrical (approximately 30 by 60 arc seconds with the long axis running NE-SW) and is displaced slightly to the NW of the central pulsar. There seems to be no evidence for a change of size at different photon energies.

Dr Peter Davison (University College, London) discussed two occultations viewed from the Copernicus satellite. The occultation paths, predicted by Dr Leslie Morrison (Royal Greenwich Observatory), are sweeping further south each month, and Copernicus was able to pass through the occultation shadow twice on October 7. In the MIT occultation, the difference in position angle of the Moon's limb on disappearance and reappearance was about 104°, giving two almost orthogonal scans, whereas for Copernicus the difference was 135°, preventing an easy study of asymmetry. The four measurements, this time in the 2.5-7.5 keV band, indicate a size of about 60-70 arc seconds. There is no evidence for the 2.5-7.5 keV emission being centred away from the pulsar position.

At least two more balloon flights and two rocket flights are to be launched and it is hoped that these, with their larger detecting areas, will be able to find details in the structure of the nebula and possibly an unpulsed flux from the pulsar itself.

Dr Jim Felten (Universities of Arizona and Cambridge) and Dr Bian Burn (Universities of Sydney and Cambridge) discussed the theoretical implications of occultation data. It is generally expected that the size of the nebula should decrease at higher photon energies. This is because the length of time for which an electron radiates by the synchrotron process at a given frequency varies inversely with that frequency. Thus if all the emitting electrons are accelerated in the neighbourhood of the central pulsar, only the less energetic ones, radiating at lower frequencies, can retain their energy long enough to stray far into the nebula: some theories predict the X-ray size to be 15 arc s, clearly at variance with the observations. The distribution of X-ray

emission across the nebula should also help to indicate the size of the region in which electron acceleration takes place. To complete the morning, Dr Jacob Shaham (Hebrew University, Jerusalem and University of Cambridge) discussed the possibility of steady X-ray emission from the pulsar itself produced by the release of elastic strain energy created by the slowing down of the pulsar's spin rate.

The afternoon session was devoted to a discussion of cosmic y-ray bursts. Dr Rodney Hillier (University of Bristol) gave a brief resume of the observations so far and Dr Arthur Bewick (Imperial College, London) reported on a possible detection of another event, made from a balloonborne detector on September 27. The original discovery of the bursts was made by the Vela satellites each of which contains a small omnidirectional γ-ray detector for the purpose of detecting y rays from nuclear explosions on Earth. The relative times at which each satellite is triggered can then be used to compute the position of the y-ray source. Serendipity again came to the aid of science when it was found that the positions of the sources of some bursts of y rays were not compatible with a terrestrial origin. Other satellites soon verified this conclusion. The total energy received in each burst is about 10<sup>-4</sup> erg cm<sup>-2</sup> and the bulk of the energy is in photons of 150 keV. although the exact shape of the spectrum is uncertain. Each burst lasts for a second or so and is often followed by another burst about 5 seconds later. Structure is evident in the data on shorter time scales as well.

The talks on observations were followed by a discussion by Professor Martin Rees (University of Cambridge) of the theoretical interpretation of these observations. Because of the relative lack of data, theorists have yielded to temptation and have had a field day constructing models to produce cosmic γ-ray burst's. There is now almost one theory for each of the twenty or so bursts seen. The power needed to produce each burst is 1033 d2 ergs, where d is the distance to the source in parsecs. Since there is as yet no clear evidence for anisotropy of the directions from which the bursts originate, it is usually assumed that their origin is either extragalactic or close by in our own Galaxy. Ideas for extragalactic sources include the early stages of radio outbursts in quasars and the outgoing shock wave produced by the explosion of a supernova. Most exponents of the theories involving nearby favour neutron stars to produce the energy needed and the short timescale: other suggestions involve scaled up solar flares, possibly from highly magnetic stars, relativistic interstellar grains

and exploding mini black holes.

Old neutron stars are thought to be quite common in the Galaxy and there are two basic means of extracting the required energy from them. First, intermittent accretion either of matter from a companion star or of comet-like bodies could explain the observed bursts. Second, starquakes can release a large amount of energy in a millisecond or so, which can be converted to y rays either by exciting particles in the star's magnetosphere or, possibly, by dissipation of seismic waves at the stellar surface. In the latter case, it was suggested that observation of nuclear lines from surface nuclei is a possibility. Future observations of these fascinating events should provide a testing ground for this plethora of theories, and should in any case (one hopes) reduce the theory-to-event ratio.

# Vertebrate palaeontology and morphology

from Barry Cox

MUCH current work in vértebrate palaeontology proceeds hand in hand with continuing investigations into the life and growth of living vertebrates, to the benefit of workers in both fields. This is the reason for the continuing success of the annual symposia on Vertebrate Palaeontology and Comparative Anatomy, the 22nd of which was held at the University of Manchester on September 24–26.

The Devonian Gogo Formation of Australia continues to produce a wealth of information, not only because it provides a new early fish fauna, but also because the specimens can be completely freed of matrix to produce undistorted three-dimensional skulls. For example, the Gogo lungfish skulls shown by R. S. Miles (British Museum, Natural History) show clearly the presence of a supraotic cavity similar to that which in living lungfish and amphibians contains an extensive endolymphatic system. Similarly, the Gogo actinopterygian skulls shown by B. G. Gardiner (Queen Elizabeth College, London) provide new evidence that the myodome evolved within that group.

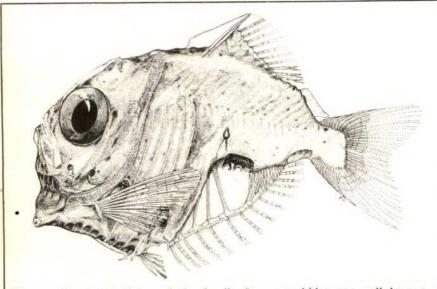
Better understanding of actinopterygian evolution, together with newer methods of phyletic investigation, are bringing more and more order to the taxonomy of the group. What had previously seemed to be taxonomically significant characters are now seen to be primitive, superficial or misinterpreted. C. Patterson (British Museum, Natural History) showed how such misunderstandings had led to the ichthyodectiform fish being placed in a

variety of other taxa, whereas they are now seen to be an independent early teleost lineage. He also pointed out that, in the case of fossil representatives of living groups, it is difficult to use a classificatory scheme to express both their phyletic relationship and their degree of morphological similarity. Patterson suggested that these difficulties could be averted by regarding each monophyletic lineage (irrespective of its rank) as a 'plesion'. These plesions would then merely be listed in a sequence, the order of which would indicate the relative morphological simlarity of each to the living sister group.

New material was also described by P. Wellnhofer (Bavarian State Museum, Munich). The fifth specimen of Archaeopteryx, though the smallest and apparently juvenile, has the most complete skull; this shows features suggesting that it was kinetic. The accepted view that it is impossible to distinguish convergent evolution in bird osteology was found by C. J. O. Harrison (British Museum, Natural History, Tring) to be unfounded—somewhat to his own surprise.

Two well known morphological/ taxonomic features received attention. The function of the hooked fifth metatarsal of lizards was explained by P. L. Robinson (University College, London). Because their proximal tarsals are functionally united with the tibia and fibula, a lever-arm for the gastrocnemius muscle cannot be formed from the calcaneum (as in mammals), but is instead provided by a tubercle on the fifth metatarsal. This fifth digit can also be rotated outwards to provide lateral stability for the foot. A new approach to the old puzzle of the differing composition of the anterior wall of the braincase in Monotremes and in other mammals was suggested by R. Presley (University College, Cardiff). who showed embryological evidence that in the latter this area may be of composite origin, including elements of both epipterygaid and prootic.

Several contributions involved biogeographical data. P. J. Miller (University of Bristol) suggested that the existence of freshwater derivatives of the marine gobies in such river systems as the Po and Arno might have been because the partial drying up, and resulting hypersalinity, of the Mediterranean during the Miocene put a premium on such evolutionary escape strategies. G. L. Underwood (City of London Polytechnic) explained how analysis of various features of the genera of natricine snakes correlated well with their distribution, spreading out from Asia through the islands of the south-west Pacific. C. B. Cox (King's College, London) showed that it was possible to define a series of



The marine hatchetfishes of the family Sternoptychidae are well known, if mainly for their rather bizarre body form and the generous development of light organs. Three genera are recognised, containing the 25 or so known species, of which Sternoptyx diaphana (illustrated) is one of the most widely distributed. Despite several studies of their biology and life style the relationships of the hatchetfishes were not well understood until a recent study by Stanley H. Weitzman (Bulletin of the American Museum of Natural History, 153 (3), 331–473; 1974) clarified their placement considerably. By detailed osteological study of several species in each hatchetfish genus as well as of their nearest relatives, Weitzmann demonstrates that they are closest to the gonostomatids. With that family, they form one of the major groups of the stomiatoid fishes. Weitzman's rearrangement of the hatchetfishes and their allies is the first to be based on osteological study of adequate material and represents a major advance in the classification of these fishes.

different patterns of faunal regions during the Mesozoic, and that these correlated well with current ideas of the varying patterns of sea barriers due partly to continental drift and partly to marine transgressions.

An elegant synthesis of electromyographic recording and of X-ray ciné film was used by K. Hiiemae (Guy's Hospital Medical School) to analyse the interrelationships between jaw movement and muscle contraction in the opossum.

## When particles hit nuclei

from D. J. Miller

How are excited states transmitted through nuclei? For some years it has been clear that we do not know the answer to this question. Surprising results were seen first in coherent production processes, where a beam particle is excited into a low-mass cluster of fast particles, without exciting or breaking the nuclear target. It seems that the fast cluster as a whole has just the same chance of interacting again, on its way out of the nucleus where it is produced, as would a single particle. This applies to clusters of two, three or five fast particles, produced by beams of gamma rays, mesons or nucleons on nuclear targets from deuterium to

Recent results from the Fermilab (Fermi National Accelerator Laboratory) near Chicago have extended our knowledge of these effects, though there is still no clear explanation. A topical meeting on high energy collisions involving nuclei was held in September at the International Centre for Theoretical Physics in Trieste. New results were reported on a whole range of related topics, including data on the multiplicity of secondary particles in particle-nucleus collisions. Nuclear emulsion workers have shown that the multiplicity of fast charged tracks produced in 200 GeV proton-nucleus collisions is only a modest factor larger than the multiplicity in proton-proton collisions at the same energy. For the mixture of nuclei in photographic emulsion, including a significant amount of bromine and silver, the factor is 1.7, and it has this value from about 70 GeV all the way up to 400 GeV. Busza's group at the Massachusetts Institute of Technology, with a simple Cerenkov counter experiment at Fermilab, has shown that the multiplicity of fast charged particles increases slowly with the atomic mass number A of the nuclide in the target, though the multiplicity in the very forward direction, within 3.5° of the beam direction, is independent of A. These experiments are 'inclusive'; they study

all final states, including the vast majority where the target nucleus has been broken up, in contrast to the unbroken nucleus involved in coherent production.

Tentative theoretical explanations by Gottfried (Phys. Rev. Lett., 32, 957; 1974), Goldhaber (Phys. Rev. Lett., 33, 47; 1974) and others have stressed the importance of the 'time dilation' effect of special relativity in understanding the passage of an excited lump of matter through a nucleus. At 200 GeV it takes about 10<sup>-23</sup> seconds for a particle to get out of a nucleus. But it may take ten times as long for a fast cluster of strongly interacting particles to get out of range of their mutual interaction. The coherent-production data show that the fast cluster behaves like one particle while it is still within the mutual interaction range. The data on inclusive multiplicities seem to be saying the same thing but with the added inference that the decay properties of the cluster are not modified by multiple collisions in nuclear matter. The results so far are fascinating, but there is scope for many more detailed measurements to be made. These could give a new source of evidence on the basic mechanisms of particle scattering, and on the structure of the particles.

# Changing views of mantle viscosity

from Peter J. Smith

THE question of the Earth's internal viscosity first became prominent in connection with vertical movements of the crust, in particular with the process of isostatic readjustment. More recently, however, the problem has assumed a much greater significance because of the important part that mantle flow processes are thought to play in the large-scale horizontal motions of continental drift and seafloor spreading. The need to find a mechanism for such motions revived the popularity of the concept of the Earth as rigid lithosphere, viscous asthenosphere, rather more viscous mesosphere and fluid core (as opposed to the equally valid, but apparently less appropriate, division into crust, mantle and core). This emphasis on viscosity then led to much closer examination of the flow properties of the asthenosphere (particularly) and mesosphere, although there is still wide disagreement over the numerical values of viscosity.

In the early studies of isostatic recovery carried out by Haskell (Am. J. Sci., 33, 22; 1937), Vening Meinesz (Proc. Kon. Ned. Akad. Wetensch., 40, 654; 1937) and others with reference to the uplift of Fennoscandia, it was assumed that the mantle flows as a

Newtonian fluid and has a uniform viscosity. This work led to the conclusion that, for an infinitely deep medium, the relaxation time (the time taken for the deviation from isostatic equilibrium to decrease to 1/e of its initial value) is proportional to viscosity but inversely proportional to the linear dimension of the removed load. Unfortunately, when Crittenden geophys. Res., 68, 5517; 1963) came to analyse the uplift of Lake Bonneville, he found that the linear dimension differed from that of Fennoscandia by a factor of about 10 but that the relaxation times for the two areas were comparable. By this time, Takeuchi (J. geophys. Res., 68, 2357; 1963) had already offered a possible explanation of the discrepancy in his suggestion that flow may be concentrated in a thin layer in the upper mantle, basing his argument on the prior conclusion by Jeffreys (Geophys. J., 8, 196; 1952) that such layered flow would involve a quite different relationship between relaxation time and linear dimension. But this was not entirely satisfactory insofar as the isostatic processes in Lake Bonneville and Fennoscandia were only reconciled by the ad hoc assumption that the flow layers beneath the two areas differ in thickness.

As a result, McConnell (J. geophys. Res., 73, 7089; 1968) decided that the assumption of uniform viscosity must be discarded, and proposed instead a mantle model comprising layers of different viscosity, generally increasing with depth. In the mean time, Gordon (J. geophys. Res., 70, 2413; 1965) had obtained a broadly similar variation of viscosity with depth by considering flow in the mantle to be due to diffusion creep (Herring-Nabarro creep). Both models led to lower mantle viscosities comparable to the 1078 poise obtained by Macdonald (Space Sci. Rev., 2, 273; 1963) from his analysis of the response of the Earth's shape to the decrease in rotational velocity. Moreover, neither model gave any cause to suppose that the assumption of Newtonian flow need be rejected; on the contrary, in Gordon's model, Newtonian flow was apparently required by the fact that for diffusion creep, stress is proportional to strain (at least for small strains).

But this consistency and agreement was soon questioned by Weertman (Rev. Geophys. Space Phys.. 8, 145; 1970) who concluded that whereas diffusion creep dominates at very low stresses (lower than about 10<sup>-2</sup> bar), at higher stresses dislocation motion becomes important. Since dislocation motion involves a non-linear stress-strain relationship, the effect of Weertman's conclusion was to overthrow the idea of Newtonian flow in the mantle. His analysis also revealed

a much slower variation of viscosity with depth than that derived from diffusion creep. Specifically, lower mantle viscosity was no higher than about 10<sup>23</sup> poise, or several orders of magnitude lower than that given by Macdonald. This was not too significant in itself, for Dicke (*J. geophys. Res.*, 74, 5895; 1969) had recently obtained a lower mantle viscosity of 10<sup>22</sup> poise from an analysis of the equatorial bulge and Goldreich and Toomre (*J. geophys. Res.*, 74, 2555; 1969) had suggested a value of 10<sup>22</sup>-10<sup>24</sup> poise to explain polar wandering.

But as Brennen (J. geophys. Res., 79, 3993; 1974) now points out, the more important problem is that the Weertman curve is apparently inconsistent with the original isostatic recovery data upon which McConnell's model was based. To discover whether the theoretical model of Weertman may be reconciled with the observational isostatic data, Brennen has thus carried out a hydrodynamic analysis of isostatic recovery flow in the mantle. He concludes, first, that the rapid increase in viscosity with depth demonstrated by McConnell and others is simply a spurious effect resulting from the incorrect assumption of Newtonian flow. The real increase of viscosity is much slower and in general agreement with that predicted by Weertman; certainly the strain rates are higher than Weertman's critical value for the onset of dislocation motion. The best fit between the data and the model is then given by a mantle viscosity which increases with depth as  $\exp(5 \times 10^{-4}z)$ , where z is the depth in kilometres. In short, not only may observation and theory be reconciled, the reconciliation may be taken as evidence that the theory is substantially correct—that is, that mantle flow is non-Newtonian and arises largely from dislocation motion.

### Glassy alloys

from Robert W. Cahn

SINCE times prehistoric, metallurgists have quenched iron or steel in water to harden them. Nowadays they know that the hardness is linked to the presence of a metastable crystal form, containing more carbon in solution than iron should at ambient temperature. This crystal form, martensite, has attracted immense research attention, because the genesis, hardness and breakdown of martensite are all absorbingly interesting scientific problems.

It is surprising, then, that it was not until 1960 that anyone reflected seriously upon the possibility of improving on the fastest rate of quenching then available: dropping a sliver of steel into a bucket of water might cool it at a measly rate of perhaps 1,000° C

per second. It was always a fair guess that the faster one can quench an alloy, the less chance it has to convert to the equilibrium structure and the more likely it is to be fixed in some abnormal and potentially intriguing crystal structure. Yet no one seriously went into the matter until Pol Duwez at California Institute of Technology, in 1960, recognised that the only effective way to speed up cooling rates substantially was to start from the liquid state. He conceived the idea of blasting a small molten drop of alloy by means of a gaseous shock wave against a sloping piece of copper: the technique soon acquired, over the opposition of its fastidious inventor, the onomatopaeic designation 'splat-cooling'. The drop is atomised and then 'splatted' out into a thin liquid layer, which gives up its latent heat to the copper in a very short space of time:

We now know that splat-cooling and related techniques can generate cooling rates from 10<sup>6</sup> to as much as 10<sup>9</sup> degrees C per second. A complete new metallurgy has been created: highly supersaturated solid solutions, a whole zoo of new metastable crystal structures, and glassy alloys have all been developed. Several hundred research papers have recently been reviewed by Jones (Rep. Prog. Phys., 36, 1425; 1973): the work covers not only structures but also anomalous mechanical, electrical and magnetic properties.

Of late, there has been a sudden burst of research on the least understood of the new metallurgical species, glassy alloys. The interest stemmed primarily from the work of David Turnbull at Harvard. Turnbull is renowned for his studies on crystallisation of molten metals, in particular his classic studies on the freezing of molten microspheres. It was a natural transition for him to examine the conditions for the congealing of liquid alloys into a glassy state, uncrystallised. He adopted one of Duwez's early alloys, Pd-Si, which could readily be liquid-quenched to a glassy state, and studied its crystallisation on reheating (for example, Chen and Turnbull, Acta Met., 17, 1021; 1969). Turnbull's ideas then inspired a metallurgist, John Gilman, to more systematic studies. It had already been recognised that alloys of transition metals with metalloids were the most sluggish to crystallise and therefore aptest to form glasses. Gilman became research manager at Allied Chemical Corporation at Princeton and instituted a systematic programme of study. His colleagues, including several of Turnbull's former collaborators, examined a range of alloys based on nickel or iron or mixtures of both, with substantial additions of carbon, boron, phosphorus, silicon and aluminium. They standardised on the 'roller-

quenching' technique: a fine stream of molten alloy impinges on a pair of rollers, very rapidly rotating in contact, and a narrow continuous glassy ribbon emerges. These glasses typically have a glass transition temperature,  $T_{\rm g}$ , in the range of 650-720 K, and a crystallisation temperature, Te, some tens of degrees higher. Polk and Chen (J. Non-Cryst. Solids, 15, 165; 1974) have recently reported on the characteristics of some of these alloys. One generalisation which emerges is that the stablest glasses-that is, those with the largest vaule of  $(T_e - T_g)$ —are those which contain the lowest metalloid content consistent with glass formation. The metalloid solute seems to exert its crystallisation-inhibiting effect by a combination of simple jamming of metal atoms (because the small metalloids fill up the voids between the metal atoms) and strong bonding between solvent and solute, which inhibits displacive rearrangements. Typical compositions include Fe75P15C6Al4 and  $Fe_{38,5}Ni_{38,5}P_{18}B_2Al_3.$ 

The glassy ribbons are extremely strong, with ultimate tensile strengths typically exceeding 200,000 pounds per square inch (Americans are incurably addicted to these venerable units). Several of these alloys, notably nickelbased alloys such as Ni-P-B-C-Al, when the roller-quenching rate was high enough, combined very high strength with measurable ductility (Chen and Polk, J. Non-Cryst. Solids, 15, 174; 1974). This is a unique combination: normally very high strength goes with extreme brittleness, as with fine silicon or carbon fibres. The manufacturers expect to apply their ribbons to reinforcing functions, for instance in car tyres.

The physical mechanism underlying the limited ductility of the alloy glasses is also strikingly novel. Pampillo and Reimschuessel (J. Mater. Sci., 9, 718; 1974) have recently shown that the ribbons deform by a process akin to glide in a metallic crystal. Thin layers of alloy, inclined at 45° to the tensile axis, behave as if they were liquid and the two halves of the ribbon slide over each other. This is probably due to the incipient destruction of short-range (glassy) order and the consequential weakening of resistance to glide. Fracture initiates at several points and the cracks spread along an already defined glide plane, and eventually the cracks collide and the crystal tears apart, showing on the fracture surfaces a pattern of veins which mark the loci along which the cracks collided.

A great deal of work is now in progress on the properties, especially the mechanical behaviour, of these alloy glasses and a number of papers is expected soon in several journals, including the three cited in this survey.

# articles

# Flux and composition of micrometeoroids in the diameter range 1–10 $\mu$ m

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A comparative morphological study of microcraters observed on glassy lunar spherules, and of those created on glassy materials in the laboratory, indicates strongly that 'irons' are the major component of the total flux of micronsized micrometeoroids. This could be because of the differential influence of solar radiation pressure on 'stones' and 'irons'.

We have compared the natural microcraters which occur on glassy lunar spherules with the craters which are produced on these spherules, and on tektite and soda lime glass and quartz crystals, by the impact of hypervelocity solid microparticles from an electrostatic particle accelerator. A scanning electron microscope and an optical microscope were used to measure the depth and diameter of the craters.

### Morphology of craters

We studied a total of 71 craters on 81 light brown, glass spherules (diameters 300–350 μm) from the Apollo 15 fines of less than 1 mm diameter obtained from Spur crater (station 7) near Hadley Rille (sample No. 15301–84). More than 90% of the craters were closely circular in form (indicating near normal incidence) with diameters in the range 1–8 μm and were identified by following the criterion suggested by Hartung et al.¹. Laboratory simulation experiments have shown that at expected impact velocities (several km s<sup>-1</sup>) crater diameters are about 1–3 times larger than the dimensions of the impacting projectile. Therefore, the craters which we have studied should have been formed in micrometeoroid (particles in the micron size regime) impacts.

The crater diameter distribution indicates that the smallest craters are most abundant with a gradual reduction in occurrence with increasing size. A most intriguing result is obtained when the ratio of depth, d, to diameter, D,  $(d|D = \chi)$  of the craters is considered. A histogram of the number of craters $\phi$  within a given range of  $\chi$  (Fig. 1a) indicates that the craters divide into at least two distinct groups. The variation of  $\phi$  with  $\chi$  does not imply a relationship between  $\phi$  and d or D, as there is no correlation between  $\chi$  and d, or  $\chi$  and D. Group I, with a mean  $\chi$  (=  $\chi$ ) = 0.94, Fig. 1a, is distinctly separated from the second major group which itself may consist of two groups, one centred around  $\chi$  = 0.50 (Group II) and another centred around  $\chi$  = 0.33 (Group III) (Fig. 1a). There is some support

for considering the low  $\chi$  grouping as two separate groups: when the data is represented as a plot of d against D (Fig. 2a), three groupings can be recognised, associated with three lines

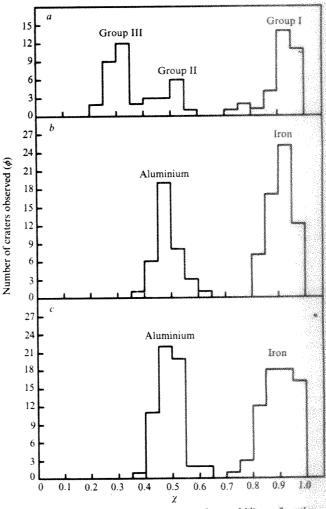


Fig. 1 Histograms of number of craters observed  $(\phi)$  as a function of the crater depth to diameter ratio  $(\chi)$  for: a, natural craters on glassy lunar spherules, showing Groups I, II and III; b, craters produced by the impact of aluminium projectiles on tektite glass and iron projectiles on glassy lunar spherules; c, craters produced by the impact of aluminium and iron projectiles on soda lime glass.

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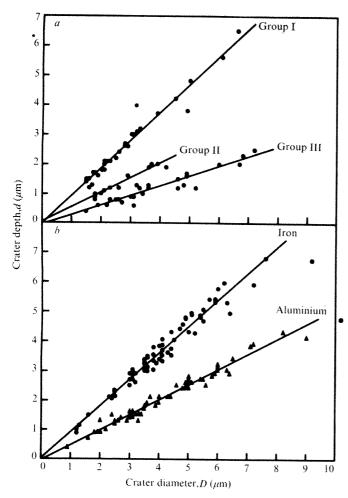


Fig. 2 Depth, d, against diameter, D. The lines are least squares fits to the data points, the slopes of which provide a value for  $\overline{\chi}$ . d, Natural craters on glassy lunar spherules, showing crater Groups I, II and III, with  $\overline{\chi}=0.94\pm0.03,\ 0.50\pm0.04$  and 0.33  $\pm0.02$ , respectively; b, craters produced by the impact of iron and aluminium projectiles on soda lime glass, with  $\overline{\chi}=0.89\pm0.02$  and 0.51  $\pm0.01$ , respectively.

of differing slope. The slopes of the least squares fits for the three sets of data provide a convenient means of expressing  $\bar{\chi}$  for each group, and are in fact those already given. The intercept of each fitted line passes closely through the zero of coordinates; when Groups II and III are considered together the fitted line has a slope smaller than either II or III separately, and a relatively large positive intercept on the d axis. We feel that that is supporting evidence in favour of two separate groups. Figure 2a also indicates that the crater diameters in Group II have lower values than either of the other two groups, but this may be because of the relatively smaller number of

samples. The average crater diameters are: Group I, 2.8  $\mu m$ ; Group II, 2.7  $\mu m$ ; Group III, 3.8  $\mu m$ .

On that basis we speculate that the three crater types either result from three groups of micrometeroids possessing quite different physical properties (for example, iron and stony types), or from three differing velocity groupings of impacting micrometeroids.

To help us to understand these results we produced craters in soda lime and tektite glasses-chosen because of their similar physical properties to the lunar glasses-by bombardment with iron and aluminium microparticles ( $d = 0.5-6 \mu m$ diameter) which were accelerated electrostatically to impact velocities of 1-7 km s<sup>-1</sup> using a 2 MV Van de Graaff generator<sup>2</sup>. Velocity selection was not available with this apparatus so the microparticle beam contained a distribution of those velocities, with a most probable velocity of about 3 km s<sup>-1</sup>. Approximately 60 craters were studied in each case and striking results were obtained when  $\phi$  was plotted against  $\chi$ . Figure 1b shows the histogram peaks for the impact of iron on lunar glass, and aluminium on tektite glass; Fig. 1c is for both iron and aluminium projectiles on soda lime glass. The correlation of the iron projectile peaks with the Group I craters is obvious; the aluminium projectile peaks correlate with the low  $\chi$  groupings, favouring the Group II natural craters. The limited spread in  $\chi$  for the different glasses suggests that these data can be compared confidently with the natural crater results. Figure 2b shows d against D for impacts of both iron and aluminium projectiles on soda lime glass; the slopes of the least squares fits through the points again provide an appropriate value for  $\bar{\chi}$ . The corresponding data for tektite and lunar glass are of equal quality; the available results for the combinations of projectile and target materials are summarised in Table I.

Iron (density 7.9 g cm<sup>-3</sup>) was chosen as an appropriate projectile material because it clearly has physical properties similar to iron meteoroids3,4 and aluminium was considered suitable because its density (2.7 g cm<sup>-3</sup>) and melting point are similar to those of the stony (chondritic) meteoroids. Lower density materials in the micron size regime are difficult to obtain but data are available in the literature<sup>5,6</sup> for the impact of micron-size polystyrene projectiles (density 1.05 g cm<sup>-3</sup>) in a comparable velocity range (2-14 km s<sup>-1</sup>) on soda lime glass. These data shows a relatively wide scatter in  $\chi$  but it has an average value of 0.22, which, taken with the present data, identifies a trend of decreasing  $\overline{\chi}$  with decreasing density of the projectile material. Mandeville has also obtained data for the impact of aluminium projectiles on soda lime glass under conditions similar to those maintained during our work, and be obtained an average value for  $\chi$  of 0.47, in close agreement with our result.

### Nature of impacting particles

Is that sufficient evidence to suggest that the three groups of natural craters originate from physically different micro-

Table 1 Data obtained for craters produced in the laboratory by the impact of microparticles of iron, aluminium and polystyrene on several target materials

	Projectile material		Iron (7.9 g cm <sup>-3</sup> )			Aluminium (2.7 g cm <sup>-3</sup> )		Polystyrene* (1.05 g cm <sup>-3</sup> )
1	Target material	Lunar spherules	Tektite glass	Soda lime glass	Quartz	Tektite glass	Soda lime glass	Soda lime glass
***************************************	D <sub>m</sub> (μm)† π ‡ χ §	3.00 2.04 0.91	3.11 2.11	4.47 3.04 0.89	2.79 1.89	3.3 1.40 0.48	4.22 1.80 0.51	4.12 1.34 0.22

<sup>\*</sup> Data for polystyrene from refs. 5 and 6.

 $D_{\rm m}$ , Mean crater diameter.

<sup>†</sup> η, Mean crater diameter to particle-diameter ratio. § χ, Mean crater depth-diameter ratio.

meteoroids? The greatest weakness of this argument is the lack of information on the functional variation of  $\chi$  with impact velocity and the doubt about whether laboratory impact velocities are appropriate to micrometeoroid lunar impacts. The concensus is that the impact velocities of lunar micrometeoroids lie in the range 5-20 km s<sup>-1</sup> (refs 1 and 7-9, and Johnson, J. H., et al., unpublished). Thus, we feel that our impact velocities are acceptable, and the small amount of information concerning the variation of  $\chi$  with impact velocity tends to show only a weak velocity dependence of  $\chi$  in the appropriate velocity range. Thus, it may well be that the broadening of the  $\phi$  against  $\chi$  histogram peaks (Fig. 1) is partly because of the finite velocity spectrum both in the natural and laboratory produced craters.

On the basis of the experimental evidence and reasoning already discussed here, we suggest that the three natural crater groupings result from the impacts of micrometeoroids of distinctly different physical properties. Group I craters with the high ₹ values—the least contentious case—we ascribe to high density particles; most probably iron micrometeoroids. Groups II and III we ascribe to the impact of low density microparticle material such as the stony and carbonaceous chondrite material, or even ice crystals<sup>10</sup>. Further deductions can be made by reference to Fig. 3 in which the densities of the laboratory iron, aluminium and polystyrene microparticles are plotted against the  $\chi$  values of the laboratory craters produced on soda lime, tektite and lunar glasses. The curve has been fitted through the origin of the coordinates. If the presented data are interpreted literally, then Group II of the natural craters would seem to be formed by micrometeoroids with effective densities in a limited range, centred about 3 g cm<sup>-3</sup>. On this evidence the Group III natural craters are apparently formed by particles with densities of 1-2 g cm<sup>-3</sup> (allowing for errors of extrapolation) and it is possible that they may result from very low density porous material ('spongy' micrometeoroids). It has often been speculated that very low density material forms a significant component of micrometeoritic material<sup>11,12</sup> and the present data suggest that about 40% of the total flux of the micrometeoroids in this particular particle size range are of this low density (Group III). We consider that of the 71 natural craters, 33 can be assigned to Group I (iron type), 12 to Group II and 26 to Group III. It follows, therefore, that about 40-50% of the particle flux is iron micrometeoroids.

An indication of the mass distribution between the three groups can be obtained if the micrometeoroid sizes can be

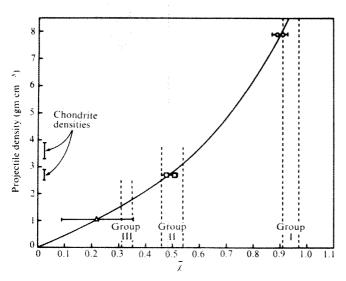


Fig. 3 Plot of projectile density against  $\widehat{\chi}$  for artificially accelerated microparticles.  $\bigcirc$ , iron;  $\bigcirc$ , aluminium;  $\triangle$ , polystyrene; from the data given in Table 1. The horizontal error bars represent the standard error of the mean. The vertical dashed lines indicate the spread in  $\chi$  of the Groups I, II and III natural craters on the lunar spherules.

estimated. Laboratory experiments have shown that the average ratio, η, of crater diameter to projectile diameter, is related to the density of the projectile material, but is essentially independent of velocity. That  $\eta$  is independent of velocity is supported by McDonnell et al.13 who studied the impact of iron projectiles on lunar rock in the velocity range 3-11 km s<sup>-1</sup>. Table I summarises the averaged values of  $\eta = \eta$  obtained for the impact of the microparticles of the three different materials on several target materials. For the glassy lunar spherules, data for iron projectiles only is available but, as can be seen (Table 1), the value of  $\eta$  is closely similar to that for the impact of iron projectiles on (the very similar) tektite glass. Therefore, we have assumed that glassy lunar spherules and tektite glass behave in the same way under bombardment by particles. Data for polystyrene projectiles are only available for soda lime glass and so we have deduced a value of  $\eta$  for polystyrene on to glassy spherules. The value of η for iron on tektite glass, and glassy spherules, is about 75% of that for iron on soda lime glass (Table 1). The same percentage also applies to aluminium projectiles on to these two target materials; itself an interesting result. We therefore assume that this same fraction applies to polystyrene projectiles on to soda lime and tektite glasses, resulting in a value of n for polystyrene onto tektite, and thus lunar glass, of about unity. Thus, we estimate that for lunar glasses the appropriate values for  $\widetilde{\eta}$  are:  $\widetilde{\eta}_{Fe}\approx 2.1;\ \widetilde{\eta}_{Al}\approx 1.4;\ \widetilde{\eta}_{poly}\approx 1.0.$  For these deduced ratios we estimate that the mean micrometeoroid diameters,  $D_m$ , are: Group I,  $D_m = 1.3 \mu m$ ; Group II,  $D_m = 2.0 \, \mu \text{m}$ ; Group III,  $D_m = 3.8 \, \mu \text{m}$ . It follows, therefore, that the mass distribution can be estimated.

The result indicates that about 20% of the total mass of the micrometeoroid material is associated with Group I (iron) micrometeoroids, and that the remaining 80% is divided between the low density (stony) Groups: II (10%) and III (70%). That is not an unreasonable result when compared with other estimates of the meteoric material in the Solar System<sup>4</sup>. That the large percentage of the mass of micrometeoritic material apparently exists in the low density form is, however, of considerable interest, although the results indicate that the density does not fall towards the very low densities inferred by Verniani<sup>2</sup> who observed radio meteors (mean density  $\approx 0.3$  g cm<sup>-3</sup>). Radio meteors are, however, substantially larger, and it does not seem unreasonable to speculate that if such relatively large pieces of 'spongy' material disintegrate to produce smaller micrometeoroids, then somewhat higher density particles may result. It should be noted, however, that very shallow microcraters ( $\chi \leq 0.1$ ) would be very difficult to recognise using our techniques, so craters produced by very low density material may be undetectable.

It could be argued that the low  $\chi$  Group (III) craters are formed by projectiles of lunar origin, that is, ejecta fragments with velocities lower than the lunar escape velocity,  $\leq 2 \text{ km s}^{-1}$ . Hartung et al. have discussed in some detail the possible role of ejecta and they concluded on the basis of estimated relative frequencies of impact and velocity spectra of both ejecta fragments and micrometeoroids, that most microcraters, especially those containing melted host material (the type of craters we examined) are attributable to the impact of micrometeoroids. Also, no spherules with diameters smaller than several tens of microns have been found in these lunar soils, which suggests that ejecta of micron size is not produced in significant amounts. We therefore conclude that Group III craters are caused by micrometeoroids.

In similar studies of depth-diameter ratio Brownlee et al.\* apparently obtained significantly different results. They studied 68 craters with diameters of 0.2-100  $\mu$ m and found no obvious dependence of  $\chi$  on crater diameter (as in all of our studies) They did, however, find a larger dispersion in  $\chi$  among the smaller craters (< 4  $\mu$ m), but they suggested that this may have been because of increased measurement difficulties for the smaller features. They therefore considered the depth-diameter ratio of only those craters with diameters greater

than 4  $\mu$ m (34 craters), and the resulting histogram of  $\phi$  against  $\chi$  shows an obvious peak at  $\chi=0.7$ . They concluded that this represented evidence against significant numbers of micrometeoroids with densities as high as iron; the majority of particles had densities of 2-4 g cm<sup>-3</sup>. They also concluded that the absence of very shallow craters ( $\chi<0.2$ ) suggests that projectiles of very low density (<1 g cm<sup>-3</sup>) are nonexistent or very rare. This very significant result is in complete accord with our data (Fig. 1a) but is in sharp contrast to most meteoroid deceleration data<sup>12</sup>. Their major deduction concerning the density of microparticles seems at variance with our result. A plausible explanation of this apparent discrepancy is, however, possible when it is seen that the two sets of data refer to significantly different mean crater diameters (our work: 3  $\mu$ m; Brownlee et al.: 19  $\mu$ m) and we are, therefore, dealing

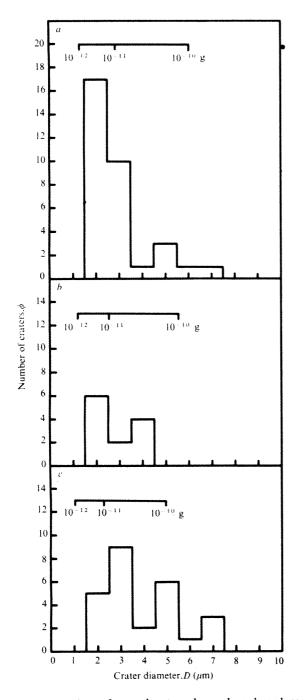


Fig. 4 The numbers of natural craters observed on glassy lunar spherules as a function of the crater diameter for Groups I, II and III (Fig. 1). The equivalent micrometeoroid masses calculated from the inferred material densities are indicated for each group. a, Group I  $(\overline{\chi}=0.94)$ ; b, Group II  $(\overline{\chi}=0.50)$ ; c, Group III  $(\overline{\chi}=0.33)$ .

**Table 2** Data relating to the natural microcraters observed on the sample of glassy lunar spherules

Number of craters observed $\frac{D_{m}}{\chi}$ (µm)  Deduced density (g cm <sup>-3</sup> ) Deduced $\eta$	Group I	Group II	Group III
	33	12	26
	2.8	2.7	3.8
	0.94	0.50	0.33
	8	3	1-2
	2.1	1.4	1.0
Deduced range of particle diameters (µm) Deduced mass range (g × 10 <sup>12</sup> )	0.7–3.1	1.3-2.9	1.5-7.2
	1.5–130	3.3-36	2.7-290

with micrometeoroids in quite separate size and mass ranges. Using an  $\tilde{\eta}$  of 1.4 we estimate that the average micrometeoroid size appropriate to the Brownlee *et al.* data is about 14  $\mu$ m, and so in the case of the low density particles (2–4 g cm<sup>-3</sup>) we are dealing with an average difference in mass of about 100 times between the two sets of data.

Figure 4 shows the  $\phi$  against D histograms for the three groups of natural craters. A very rapid increase in  $\phi$  with decreasing D is apparent for the Group I iron micrometeoroids (Fig. 4a). This, coupled with the low density Group III data (Fig. 4c) which, within the statistical fluctuation, indicate a much slower increase in  $\phi$  with decreasing D, strongly suggests an increasing contribution of iron type micrometeoroids to the total micrometeoroid flux in the micron size region. Little can be said about the Group II data (Fig. 4b) except that they do not seem to conflict with the general conclusions drawn for the other two groups. Accordingly, we suggest that the nature of micrometeoritic material is strongly dependent on the region of the mass spectrum being considered, the proportion of low density stony material decreasing and the proportion of high density iron particles increasing with decreasing particle size in the 0.5-8 µm diameter range. This conclusion is most forcibly demonstrated in Fig. 5 in which the absolute differential fluxes of the Groups I and III micrometeoroids are plotted against mean particle diameter. The three points on each curve were obtained by summing the total number of craters observed in the intervals of crater diameter 1.5-3.5  $\mu m$ , 3.5-5.5  $\mu m$ , and 5.5-7.5 µm, and deducing the mean particle diameters using the appropriate values of  $\bar{\eta}$  (Table 1). The absolute differential flux was obtained from a consideration of the total number of particles observed in each group, the scanned area of the lunar spherules and the estimated exposure time to micrometeoroid bombardment. The last was estimated using the technique<sup>14</sup> of charged particle track analysis. The detailed form of the two curves cannot be interpreted too literally because of the limited amount of data from which they were derived. We believe, however, that the gross features are significant, and indicate that for a particle diameter in excess of about 2 µm, the major fraction of the micrometeoroid flux consists of low density material. The reverse seems to be the case for particle diameters approaching 1 µm, where the high density microparticles predominate. This most interesting feature of the data is quite consistent with the often predicted, but to our knowledge never observed, effects of solar radiation pressure, which acts against solar gravity to exclude very small particles from the Solar system. This pressure is differential in that it acts to a greater extent on low density materials and is thus expected to influence the flux of the Group III micrometeoroids to a greater extent than those of Group I. The magnitude of the radiation pressure effect is traditionally referenced to the particle mass for a given density of material, and so the equivalent mass scales for the two groups-which are not common because of the different material densities of the two groupsare also included in Fig. 5. These results were obtained from simple classical theory<sup>15</sup>. Thus, for 2 µm diameter particles the effect is predictably severe on the Group III particles and the reducing slope of the Group III curve of Fig. 5 probably reflects the increasing effect with decreasing size (mass). The

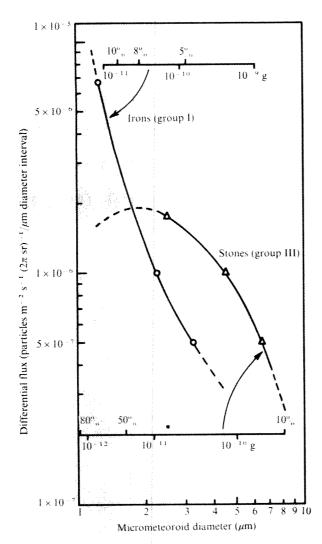


Fig. 5 Differential micrometeroid flux as a function of micrometeroid diameter for irons (Group I) and stones (Group III). The equivalent mass scale and a percentage index of the effect of solar radiation pressure are also indicated for each group. The percentages indicate the magnitude of the outward force resulting from solar radiation, relative to that of solar gravity at 1 AU. Thus, at 50% the former is half of the latter.

extrapolation of the curve (dashed) is simply a recognition of the fact that when the radiation pressure effect exceeds 100% (that is, exceeds gravity) then a 'cut off' results and no particles below a given mass ( $\approx 4.10^{-13}$  g) or diameter (0.8 µm for a material density of 1.5 g cm<sup>-3</sup>) can exist in stable orbits in the Solar System. For Group I particles of 2 µm diameter, radiation pressure effects are small (a few percent) and thus the differential flux of iron micrometeoroids continues to increase even at very small particle diameters, until the classical 'cut off' at about  $0.2 \, \mu m \, (2.10^{-14} \, g)$  is approached.

The number of craters which we observed per cm<sup>2</sup> of the lunar glasses agrees well with that of Brownlee et al.9: (both samples were from the Apollo 15 sites). We found approximately  $3\times10^3$  craters cm  $^{-2}$  in the diameter range 1.5–7.2  $\mu m$ whereas the Brownlee et al. found  $7 \times 10^3$  cm<sup>-2</sup> in the same diameter range. They found an increasing incidence of small craters with decreasing crater diameter, with the cumulative crater surface number density approximately  $5 \times 10^4$  cm<sup>-2</sup> at a crater diameter of 0.3  $\mu$ m. An extrapolation (on a log  $\phi$  against log D plot) of our Group I data (Fig. 4a) to a crater diameter of 0.3 µm (that is, to particle diameters near to the classical solar radiation pressure limit) indicates a cumulative number density of  $\sim 7 \times 10^4$  craters cm<sup>-2</sup>. The similarity of these estimates tends to indicate that the smaller features in the Brownlee et al. data were produced by the impact of iron

micrometeoroids, because similar extrapolation of the Group III data (stony types) indicates much smaller number densities for crater diameters of less than 1 μm.

Of much greater interest in meteorite studies is the cumulative flux of micrometeoroids at any given mass, and how this parameter varies with mass. The cumulative flux of all micrometeoroids above a mass of 10<sup>-11</sup> g is indicated by the present data to be approximately  $2 \times 10^{-5} \, \text{m}^{-2} \, \text{s}^{-1} \, (2\pi \, \text{sr})^{-1}$  using the derived exposure time to meteorite impact of ~ 5.104 yr (ref. 14). This result compares very favourably with the satellite data of Alexander et al. reported by Hughes16, which suggests a value of  $\sim 5 \times 10^{-5} \text{ m}^{-2} \text{ s}^{-1} (2\pi \text{ sr})^{-1}$  at  $10^{-11} \text{ g}$ . The very great difference in the effective sampling time between the satellite experiments (~ 1 yr) and the present 'crater technique' seems to suggest that the micrometeoroid flux has remained sensibly time invariant during the recent history of the Solar System.

A further important result is obtained if the cumulative crater data at the classical solar radiation pressure limit (obtained by applying the extrapolation procedure to the Group I data) is converted to cumulative micrometeoroid flux. The value thus indicated at a micrometeoroid mass of 10<sup>-14</sup> g is approximately  $10^{-3}$  m<sup>-2</sup> s<sup>-1</sup>  $(2\pi$  sr)<sup>-1</sup>. If the mass limit of solar radiation pressure for iron micrometeoroids were lower than that predicted by the classical theory, then it is likely that relatively large fluxes of very small iron micrometeoroids ( $d < 0.1 \mu m$ ) could exist in stable orbits in the Solar System. Highly sensitive detectors would be necessary to verify the existence of such small particles, such as the plasma micrometeoroid detector<sup>47</sup> incorporated into the Prospero satellite18 which did, in fact, indicate a total cumulative flux at its predicted threshold mass sensitivity of  $10^{-15}$  g in the range  $10^{-2}$  to  $10^{-1}$  m<sup>-2</sup> s<sup>-1</sup>  $(2\pi$  sr)<sup>-1</sup>.

Our study indicates that the differential flux of iron type micrometeoroids increases rapidly as their size reduces progressively to diameters below about 2 µm. For micrometeoroid diameters  $\geq 2 \mu m$ , micrometeoroids with a density of 1-4 g cm<sup>-3</sup> increasingly contribute to the flux, until they represent in excess of 90% of the total. The available evidence indicates that the major component of the flux at this low density grouping is of material in the density range 1-2 g cm with a smaller component at a density near to 3 g cm<sup>-3</sup>. Thus, the data seem to support the accepted view that iron type meteoroids represent only a very small fraction of the total mass of meteoritic material. In the diameter range 0.1 µm-1.0 µm, however, a large fraction of the total flux of micrometeoroids must be of the high density iron type and their cumulative flux at the lowest mass may approach the relatively high values indicated by the most sensitive micrometeoroid detectors.

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### Partial nucleotide sequence of a prokaryote initiator tRNA that functions in its non-formylated form

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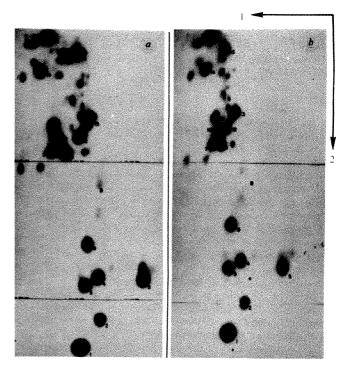
When grown in the absence of folate, Streptococcus faecalis initiates protein synthesis with non-formylated . methionyl-tRNA1 Met. The nucleotide sequence of this tRNA differs from that of tRNAi Met synthesised in the presence of folate — which functions as N-formylmethionyl-tRNA: Met—only in the 'GT\psiC' loop, where uracil is found in the tRNAi Met of folate-free cells in place of thymine.

Initiation of protein synthesis in the bacterium Streptococcus faecalis R is an interesting exception to the generalisation concerning the difference in this process in prokaryotes and eukaryotes. Initiation of protein synthesis in all prokaryotes and certain organelles of eukaryotes appears to involve a special tRNA, tRNA, that functions as a formylated methionylated derivative, N-formyl-methionyl $tRNA_{\rm f}^{\rm Met}$ , and donates a formyl-methionyl residue to the N-terminus of the peptide chain<sup>1-3</sup>. The formyl group essential for the initiation process is transferred to methionyltRNA<sub>f</sub><sup>Met</sup> from a derivative of folic acid, 10-formyltetrahydrofolate, by means of a specific enzyme<sup>4,5</sup>. Initiation of protein synthesis in the cytoplasm of eukaryotes also involves a unique tRNA Met species. However, formylation of eukaryotic methionyl-tRNA, Met does not occur in vivo, although methionyl-tRNA $_{i}^{Met}$  from some eukaryotes can be formylated in vitro by the transformylase of Escherichia coli³.

S. faecalis cannot synthesise folate or its derivatives de novo and normally requires the presence of one of these substances in the growth medium. The organism, however, can grow at essentially the normal rate in the absence of any of these compounds when the growth medium contains serine, methionine, thymine, a purine base and pantothenate6, each substance a product of a synthetic pathway involving a folate derivative7.8. N-formyl-methionyl $tRNA_f^{Met}$ , considered to be essential for initiation of protein synthesis in bacteria<sup>1-3</sup>, is also a product of an enzymatic reaction involving a folate derivative4.5. However, it is not necessary to supply the organism with this substance when it is grown in folate-free medium. Samuel et al.9-11 demonstrated that under these growth conditions, initiation of protein synthesis proceeds with non-formylated methionyl-tRNA<sub>f</sub><sup>Met</sup> and appears in this respect to resemble the protein initiation process in eukaryotes. Their data showed that the ability of this bacterium to initiate protein synthesis with non-formylated methionyl-tRNA<sub>f</sub><sup>Met</sup> is attributable to an alteration in the tRNA<sub>t</sub>Met, and not to a modification of the ribosomes or factors involved in protein initiation. The tRNA<sub>f</sub>Met of S. faecalis grown in the absence

of folate can be distinguished from the tRNA<sub>1</sub>Met of S. faecalis grown in the presence of folate by several physical, chemical and biological criteria. Of particular significance is the observation that non-formylated methionyl-t $RNA_f^{Met}$ from minus-folate cells forms an initiation complex with ribosomes, poly (A,U,G), and initiation factors under conditions where methionyl-tRNA<sub>1</sub>Met from plus-folate S. faecalis, like other prokaryotic initiator tRNAs, requires prior formylation for formation of the initiation complex<sup>11</sup>.

Sequence analyses have been undertaken in several laboratories in efforts to identify structural features of the tRNA Met species that are responsible for their different biological properties. The nucleotide sequences of two isospecies of tRNA<sub>f</sub><sup>Met</sup> and two of tRNA<sub>Met</sub> from E. coli have been determined<sup>12-14</sup>. More recently the sequences of the tRNA<sub>1</sub>Met from yeast<sup>15</sup> and three mammalian sources<sup>16,17</sup> have been reported. The tRNA<sub>f</sub>Met sequences of the mam-



Autoradiograph of the two-dimensional fractiona-Fig. 1 Autoradiograph of the two-dimensional fractionation of the U<sub>1</sub> RNase digestion products of (a) plus-folate and (b) minus-folate S. faecalis tRNA<sub>1</sub><sup>Met</sup>. (1) First dimension: cellulose acetate, pH 3.5; (2) second: DEAE-cellulose, 7% formic acid. B indicates position of standard blue marker<sup>18</sup>.

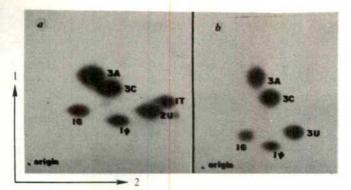


Fig. 2 Two-dimensional fractionation of 3' nucleotides obtained by combined pancreatic RNase and T<sub>2</sub> RNase digestion of oligonucleotide U-16 from (a) plus-folate and (b) minus-folate S. faecalis tRNA<sub>1</sub><sup>Met</sup>. (1) First dimension: isobutyric acid-NH<sub>4</sub>OH; (2) second: isopropanol-HCl-H<sub>2</sub>OI<sup>9</sup>. Bases are identified by standard letter designations with a numeral indicating the number of equivalents found.

malian cells are identical and show a high degree of homology with  $E.\ coli\ tRNA_1^{\rm Met}$ , as well as with yeast  $tRNA_1^{\rm Met}$ , that is suggested to reflect the special constraints in evolutionary divergence that the unique role of this tRNA has imposed. An unusual feature of eukaryote  $tRNA_1^{\rm Met}$  is the lack of a  $GT\psi C$  sequence in loop  $IV^{1s-17}$ . It was thus of particular interest to note that the  $tRNA_1^{\rm Met}$  of  $S.\ faecalis$  grown in the absence of folate does not contain ribothymidine (T), whereas  $tRNA_1^{\rm Met}$  from cells grown with folate does contain this modified nucleoside. We have therefore investigated the more detailed chemical structure of  $tRNA_1^{\rm Met}$  of  $S.\ faecalis$ .

S. faecalis was grown in the presence (plus-folate) or in the absence (minus-folate) of folic acid in a low phosphate medium containing <sup>32</sup>P-phosphate, a modification of a medium described previously <sup>30</sup>. Cells were collected by centrifugation and then broken by sonication. Unlabelled carrier tRNA was added and the tRNA<sub>f</sub> <sup>Met</sup> was isolated and purified essentially as described elsewhere <sup>31</sup>. The final tRNA<sub>f</sub> <sup>Met</sup> sample was estimated to be 80–90% pure.

The tRNA<sub>1</sub><sup>Met</sup> was analysed as described by Barrell<sup>18</sup>, except that U<sub>1</sub> RNase, a gift of Drs A. Blank and C. A. Dekker, was used instead of T<sub>1</sub> RNase and frequent use was made of the two-dimensional thin-layer chromatography system for nucleotides described by Nishimura<sup>19</sup>. The detailed data of this investigation will appear elsewhere. As suggested by Barrell<sup>18</sup>, except where stated otherwise, the letters A, C, G, and U are used for the 3' nucleotide residues and a sequence such as ACG represents ApCpGp. Parentheses enclosing letters indicate that the sequence of these nucleotides is unknown.

Two-dimensional thin-layer chromatography<sup>19</sup> of this 3' nucleotides obtained on enzymatic digestion showed that, in addition to the four major nucleosides, plus-folate  $tRNA_I^{Met}$  contains one T, one pseudouridine ( $\psi$ ), and one dihydrouridine (hU), whereas minus-folate  $tRNA_I^{Met}$  contains only one  $\psi$  and one hU.

As Fig. 1 shows, the fingerprints of the oligonucleotides obtained by digestion of plus-folate and minus-folate  $tRNA_1^{Met}$  with  $U_1$  RNase were essentially identical, indicating that the sequence of the two tRNAs are similar. Analysis of the oligonucleotides showed that the compositions of corresponding fragments were the same for both tRNAs except for oligonucleotides 16 (designated U-16) which, as seen in Fig. 2, was shown by two-dimensional thin-layer chromatography to have a composition  $(T\psi U_2C_3A_3)G$  in plus-folate  $tRNA_1^{Met}$  and  $(\psi U_3C_3A_3)G$  in minus-folate  $tRNA_1^{Met}$ , suggesting that in the latter case a single U residue had not undergone methylation to produce T (5 methyluridine).

As Fig. 3 shows, fingerprints obtained on pancreatic

RNase A digestion were also quite similar for the two tRNAs, establishing the sequence similarity. In plus-folate tRNA<sub>1</sub><sup>Met</sup>, T was found in oligonucleotide 12 (P-12), the composition of which was shown to be (AG<sub>2</sub>)T. The corresponding oligonucleotide from minus-folate tRNA<sub>1</sub><sup>Met</sup> had the expected composition (AG<sub>2</sub>)U. All other oligonucleotides examined had identical composition for the two tRNAs. Attention was thus focused on the sequence surrounding T from plus-folate tRNA<sub>1</sub><sup>Met</sup> and the corresponding sequence from the minus-folate tRNA<sub>1</sub><sup>Met</sup>.

On further digestion of the U-16 oligonucleotides with pancreatic RNase, both the plus-folate and minus-folate fragments yielded one equivalent of A3U, one equivalent of G, and three equivalents of C; however, the plus-folate sample yielded an equivalent each of T,  $\psi$ , and U, whereas the minus-folate sample gave one equivalent of  $\psi$  and two equivalents of U. On partial digestion with U2 RNase, both samples yielded (U2C2)G, A3, A2 and A fragments. However, plus-folate U-16 yielded the oligonucleotides  $(T\psi CA)$  and  $(T\psi C)$ , whereas the corresponding minus-folate fragments were  $(U\psi CA)$  and  $(U\psi C)$ . After removal of the phosphate from the unexpected but consistently obtained U2 RNase digestion product (UψC), pancreatic RNase digestion produced U and  $\psi$ , indicating that Comwas at the 3' end of the dephosphorylated fragment, this base having been released as the nucleoside and thus undetected by autoradiography. From these data one can deduce the sequence (Tψ)CAAAU(CCU)G for the oligonucleotide U-16 from plus-folate tRNA<sub>f</sub><sup>Met</sup> and (Uψ) CAAAU(CCU)G for the corresponding oligonucleotide from minus-folate tRNA<sub>f</sub>Met.

The final sequence was derived by analysis of the pancreatic RNase product P-12 from plus-folate tRNA<sub>1</sub><sup>Met</sup>, the composition of which is (AG<sub>2</sub>)T. The T is automatically assignable to the 3' end by nature of pancreatic RNase specificity. After removal of the 3' phosphate, snake venom phosphodiesterase treatment yielded pG and pT in the ratio 2: 1, thus establishing the sequence as AGGT. Since there is only one T in plus-folate tRNA<sub>1</sub><sup>Met</sup>, oligonucleotides P-12 and U-16 must overlap and T must be at the 5' end of the oligonucleotide U-16. Figure 4 shows the derived sequence of what is apparently the 'GTΨC' loop (IV) and stem of tRNA<sub>1</sub><sup>Met</sup> of S. faecalis.

This sequence bears striking resemblance to the 'GT\square\C'

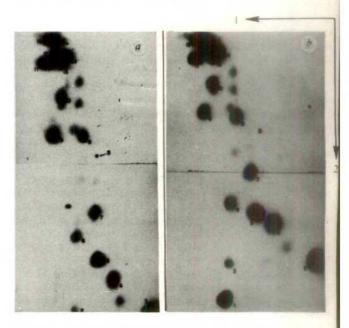


Fig. 3 Autoradiograph of two-dimensional fractionation of pancreatic RNase digestion products of (a) plus-folate and (b) minus-folate S. faecalis tRNA<sub>r</sub><sup>Met</sup>. (1) and (2) as in Fig. 1.

B indicates position of blue marker.

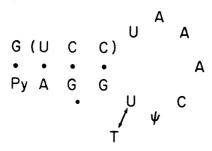


Fig. 4 Nucleotide sequence of the presumed loop IV and stem of tRNA<sub>1</sub><sup>Met</sup> from minus-folate S. faecalis (primary sequence) and tRNA<sub>1</sub><sup>Met</sup> from plus-folate cells (T replacing U).

arm of tRNA<sub>f</sub><sup>Met</sup> of E. coli<sup>13</sup>, the only difference being that in the E. coli tRNA a G-C pair is found in place of the presumed U-A base pair in the stem of the S. faecalis tRNA. Recently it has been shown that the single-stranded portion of the 'GT\(psi C'\) arm of Anacystis nidulans tRNA<sub>t</sub> Met has this same base sequence20.

The similarity in the U<sub>1</sub> RNase digestion products of the plus-folate and minus-folate tRNAs in combination with the similarity of their pancreatic RNase digestion products indicates that the remainder of the nucleotide sequence is identical for the two S. faecalis tRNAs. Although the complete sequence of tRNA<sub>1</sub>Met of S. faecalis has not been determined, a comparison of its enzymatic digestion products with those of tRNA<sub>f</sub><sup>Met</sup> of E. coli<sup>12</sup> indicates that there is a great deal of similarity between these two molecules.

Like tRNA<sub>f</sub><sup>Met</sup> of E. coli, the S. faecalis tRNA has pCG (oligonucleotide U-7) at the 5' terminus. It is not known, however, if the terminal C is hydrogen bonded to a G residue since there is uncertainty about the sequence of the 3' end of the tRNA. Oligonucleotide U-5, which has the composition (A<sub>2</sub>C<sub>3</sub>), is identifiable as the 3' terminus by its lack of G. Presumably it contains an undetectable AoH and has the sequence (CA2)CCA0H. The composition of this oligonucleotide is identical to that of the fragment CAACCA<sub>OH</sub> obtained upon T<sub>1</sub> RNase digestion of tRNA<sub>f</sub><sup>Met</sup> from E. coli<sup>12</sup>. Oligonucleotide U-5 migrates differently, however, in the second dimension from what was reported for CAACCA<sub>OH</sub><sup>12</sup>. Although the mobility of U-5 is similar to that reported for CCACCAOH20, reproducible quantitative data indicate that this is not the composition of the S. faecalis fragment. The 5' terminal residue of S. faecalis tRNA<sub>i</sub>Met, like that of tRNA<sub>i</sub>Met of E. coli<sup>13</sup> and A. nidulans20 and unlike that of most other tRNAs examined, is not hydrogen bonded to a residue four nucleotides removed from the 3' AoH. It is not known at this time, however, if the 3' terminus of S. faecalis tRNA<sub>f</sub><sup>Met</sup> is identical to or similar to that of E. coli tRNA<sub>f</sub> Met or if the 5' C is hydrogen bonded to a G that is more than four nucleotides removed from the 3' AoH.

At least a portion of the hU loop appears identical for E. coli and S. faecalis tRNA<sub>f</sub> namely, both have a single hU which occurs in the sequence GGhUAG13. (The sequence of the S. faecalis oligonucleotide P-11 is GGhU, that of U-9 is hUAG.) S. faecalis tRNA, Met on U1 RNase digestion yields (C2U)G (U-12) and on pancreatic RNase figestion AGC (P-7). These two could be from the hU loop and would be consistent with a sequence identical to that of the E. coli tRNA, namely CAGCCUGGhUAG13, the first ind last bases forming a hydrogen bond.

Oligonucleotide U-15 has the composition (A<sub>3</sub>C<sub>5</sub>U<sub>2</sub>)G and nay be from the anticodon loop since E. coli tRNA, Met on  $\Gamma_1$  RNase digestion yields the anticodon-containing oligonucleotide 2'OMeCUCAUAACCCG12. The pancreatic RNase product P-13 from S. faecalis has the sequence GGGC. These two products would be consistent with an inticodon loop and stem similar to that of the E. coli tRNA with the notable exception that tRNA; Met of S. faecalis does

not have 2'OMeC. Furthermore, although both isospecies of tRNA<sub>f</sub>Met from E. coli contain 4-thiouracil and one contains 7-methylguanine<sup>13</sup>, tRNA<sub>f</sub>Met from plus-folate S. faecalis has no modified bases other than T,  $\psi$ , and hU and tRNA<sub>1</sub>Met from cells grown in the absence of folate has only  $\psi$  and hU. S. faecalis  $tRNA_f^{Met}$  has the smallest number of modified bases observed among tRNAs.

Analysis of unfractionated and partially fractionated S. faecalis tRNA showed that the absence of T is not unique to the tRNA<sub>f</sub>Met species. Although T is present in tRNA from plus-folate cells, this modified residue is absent in all tRNA species of S. faecalis when it is grown in folate-free medium.

In summary, tRNA<sub>1</sub>Met of S. faecalis is similar in sequence to tRNA<sub>1</sub>Met of E. coli, although S. faecalis tRNA<sub>1</sub>Met lacks several modified bases found in the E. coli species. When grown in the presence of folate, S. faecalis tRNA<sub>1</sub>Met contains the modified base T in the sequence  $GT\psi C$ . When the bacterium is grown in the absence of folate, methylation of the specific U residue does not occur, the resulting sequence being  $GU\psi C$ . The remainder of the nucleotide sequence is identical for plus-folate and minus-folate S. faecalis  $tRNA_{\rm f}{}^{\rm Met}.$ 

The absence of this single methyl moiety is sufficient to alter the chromatographic and melting properties of the  $tRNA_{\rm f}^{\rm Met}$  species and, most significantly, to allow the bacterium to initiate protein synthesis with non-formylated  $methionyl\text{-}tRNA_{\mathrm{f}}^{Met}\quad when \quad 10\text{-}formyltetrahydrofolate, \quad an$ essential component of the formylation reaction, is unavailable. Further investigation is required to determine whether the ability of this tRNA, Met to function in its non-formylated form is the consequence of direct interaction of the altered portion of the tRNA with ribosomes or various factors involved in protein synthesis, or whether it is the result of a conformation change in the tRNA molecule. It would be interesting to examine the tRNA<sub>f</sub><sup>Met</sup> and protein initiation process in the mutants of E. coli which are totally lacking T21 and strains of Bacillus subtilis which are low in T content<sup>22,23</sup> to determine if the absence of T in other prokaryotic initiator tRNAs allows them to function in their non-formylated form.

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# letters to nature

#### A Newtonian view of relativistic cosmology

In spite of the success of Milne and McCrea1 in using classical mechanics to reproduce the Friedmann universes, the mathematically simplified apparatus of Newtonian cosmology has been of only limited use in exploring relativistic world models. In general, the required formulation of Newtonian universes2.3 leads to indeterminate equations of motion: one has an infinite region of homogeneous dust (density  $\rho(t)$ , pressure p=0), in which the gravitational field cannot be found classically. This letter outlines a way of resolving the indeterminacy, and gaining an insight into relativistic cosmology, through equations that reveal the dynamics of relativistic dust world models in a quasi-Newtonian fashion.

The method is a local-approximation technique using spatial power series expansions in comoving coordinates ( $\xi'$ ,  $\tau$ ). We take a cosmological metric

$$ds^2 = d\tau^2 + 2g_{4i} d\xi^i d\tau + g_{ij} d\xi^i d\xi^j$$
 (1)

and noting the conservation equations  $\partial g_{4i}/\partial \tau = 0$ , we write

$$g_{4i} = E_i + F_{ir}\xi^r + G_i^{rs}\xi^r\xi^s + \dots$$
 (2a)

$$g_{IJ} = M_{IJ} + N_{IJ}^{r} \xi^{r} + \frac{1}{2} P_{IJ}^{rs} \xi^{r} \xi^{s} + \dots$$
 (2b)

Because the coefficients on the right of (2a) are constant, there is a transformation of  $\tau$  that gives  $E_i = 0$  and  $F_{ij} = -F_{ji}$ . M, Nand P are  $\tau$ -dependent arrays, but we can set  $-M_0 = I$  (the  $3 \times 3$  identity matrix) and  $N_0 = 0$  at an initial instant  $\tau_0$ .

A local correlation is then obtained with the usual kinematics of Newtonian universes, in which the velocity v of matter, being a linear function of Cartesian coordinates  $x^{i}$ , is defined by a matrix H depending only on the absolute Newtonian time t. H decomposes into an expansion scalar H, a traceless symmetric shear tensor Q, and an antisymmetric spin tensor W:

$$\mathbf{v} = H\mathbf{x} \colon H = H(t) = \tilde{H}I + Q + W \tag{3}$$

The classical motion is given in Lagrangian coordinates by a matrix A(t). A particle initially at  $x_0$  has the general position  $\mathbf{x} = A\mathbf{x}_{\theta}$ , provided that

$$\hat{A} = HA; \ A_0 = I \tag{4}$$

We can identify the  $x_0^i$  locally with the comoving coordinates  $\xi^{i}$ , and in the local approximation we can also interchange t and τ. With these correspondences, the leading coefficients in equations (2a, b) are found to be the matrices

$$F = -A'WA; M = -A'A \tag{5}$$

This local Newtonian interpretation of a general comoving metric is useful in itself.

Although the classical potential U is indeterminate, the Euler equation shows that it may be written as a quadratic form given by a t-dependent symmetric matrix  $\Phi$ :  $U = \frac{1}{2}\Phi_{ij}x^ix^j$ ;  $\Phi = \Phi(t) = \Phi^{t}$ . The Euler, Poisson and continuity equations reduce to

$$\bar{H} + H^2 + \Phi = 0 \tag{6a}$$

$$tr(\Phi) = 4\pi G \rho$$
 (6b)

$$\dot{\rho} + \rho \operatorname{tr}(H) = 0 \tag{6c}$$

and here the inadequacy of Newtonian mechanics is obvious? we have just one equation for the six independent  $\Phi_{ij}$ . In the absence of spherical symmetry extra conditions may be imposed arbitrarily3-5, but the Φ corresponding to general relativity is far from evident.

But we can get the relativistic answer by extending the localapproximation formalism to Einstein's field equations. With the comoving coordinates chosen locally as before, it turns out that the relativistic potential matrix  $\Phi$ , is given by

$$\Phi_r = \frac{1}{2}\pi G\rho I + \overline{H}Q - (Q^2 - \frac{1}{2}tr(Q^2)I) + \Phi_c \qquad (7a)$$

$$\Phi_{c} = \psi - \frac{1}{3} tr(\psi) I; \ \psi = -(A^{-1})^{t} (\mathring{R}^{0}) A^{-1}$$
 (7b)

where the matrix  $\tilde{R}^0$  is the local value of the Ricci subtensor  $\tilde{R}_{ij}$ of the & 3-spaces. This result shows how matter, motion and spatial curvature contribute to the potential.

The determinate quasi-Newtonian potential with  $\Phi_c=0$  gives an exact description only of universes whose comoving space sections have isotropic curvature: the Bianchi Type I models, a non-rotating subclass of Type V, and the closed Friedmann universe (see ref. 6). But it is interesting that rotating solutions with  $\Phi_{
m e}=0$  need not have a singularity. (This is most easily shown for cylindrical models5.7.) We cannot infer here that similar exact relativistic solutions exist. Given the wide scope of the Penrose-Hawking theorems', the implication is rather that curvature anisotropies are a vital factor in causing singularities

In general the potential remains indeterminate, because  $\Phi_{\epsilon}$ cannot be predicted. (Though  $\Phi_c$  can be calculated for homogeneous universes, since their metrics are known in advance. Nevertheless, the quasi-classical equations are of some heuristic value, and they can be used to classify and elucidate various kinds of singularities and indefinite expansion occurring in relativistic world models. They show in particular why expand ing universes that approach isotropy have to be regarded as unusuals: the shear tensor Q is self-damping during expansion only in the special cases when  $\Phi_c$  is insignificant.

The decisive effects that  $\Phi_c$  may have are exemplified by Bianchi Type VI universes, for which solutions can be generated by computer. Some rotating models of this class exhibit no only anisotropic expansion, but also matter singularities of 'ribbon' form that seems to be hitherto unreported: the princi pal axes of a local comoving ellipsoid tend to zero, a constant and infinity. The limiting behaviour is apparently of power law type, with the volume proportional to  $\tau^p$ , where  $0 , as <math>\tau \downarrow 0$ . (In isotropic singularities p = 2, and velocity-dominated ones have p = 1 if a simple power law applies.)

These Bianchi VI singularities are distinct from the 'whimper' sort<sup>11</sup>, in which the homogeneous 3-spaces of a synchronous coordinate system change their signature. The models referred to here have been studied using a comoving metric of the form (this actually ranges over Types I, III, V, VI)

$$\begin{aligned} ds^2 &= d\tau^2 + 2\lambda \eta d\xi^2 d\tau - \{\alpha (d\xi^1)^2 + 2\nu \eta d\xi^1 d\xi^2 + \\ &+ \beta (\eta d\xi^2)^2 + \gamma (\zeta d\xi^3)^2 \} \end{aligned} \tag{8}$$

where  $\eta = \exp(m\xi^1)$ ,  $\zeta = \exp(n\xi^1)$ ;  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\nu$  are functions of  $\tau$ ;  $\lambda$ , m, n are constants. (see equations (2). The Newtonian interpretation (5) will usually require a local transformation of the metric (8).) The field equations for (8) show that only exceptional cases, if any, can pass through a whimper singularity (corresponding here to  $\alpha\beta = \nu^2$ ), and probably this is true in general.

As for matter singularities, the quasi-Newtonian equations suggest a vast range of possible behaviour in relativistic cosmology, making present observations of radiation isotropy appear all the more remarkable. Details of singularity behaviour and other specialised aspects will be discussed elsewhere.

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#### Redshifts of Markaryan galaxies

MARKARYAN<sup>1</sup> and Markaryan and Lipovetsky<sup>2</sup> have given data on a few hundred galaxies with strong ultraviolet continua. Markaryan classifies them on the basis of the concentration of the source of the continuum (s means concentrated and d diffuse objects; sd and ds are intermediate). Especially among the s-type objects there seems to be contamination with QSOs when their spectra, colour and presumable morphology (spheroidal, compact) are considered. Redshifts are now available for many Markaryan galaxies providing new material for studies of the redshift problem. It is worthwhile to see whether the claimed peculiarities in redshifts of QSOs and related emission-line objects<sup>3-6</sup> have counterparts in the distribution of these

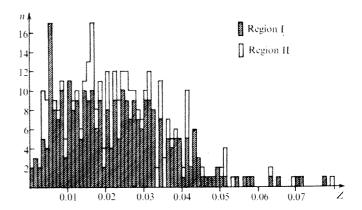


Fig. 1 Distribution of the redshifts of Markaryan galaxies.

smaller redshifts. According to Arp and Sulentic (see ref. 7) many Markaryan galaxies seem to be companions of small-redshift bright galaxies and thus they possibly have anomalous redshifts.

I present here a preliminary survey of the data which consist of 394 redshifts<sup>8-15</sup> (z < 0.08) of which 90, 92, 65, and 147 are for s, sd, ds, and d objects, respectively. The measurements of Ulrich<sup>13</sup> and Sargent<sup>14</sup> were preferred.

The distribution of all the redshifts is presented in Fig. 1, using the interval  $\Delta z = 0.001$ , corresponding to the accuracy of most redshifts. Burbidge<sup>3</sup> and Burbidge and O'Dell<sup>5,6</sup> found for different kinds of emission-line objects peaks near z = 0.03 and z = 0.06. In my analysis no groupings at these values can be seen, probably because of the different class of objects studied.

But there is a peak at z=0.015-0.016, with some interesting features. Its statistical significance can be approximated from Poisson's formula when the gross frequency distribution of the redshifts is taken as rectangular between z=0.003 and z=0.030. The probability that 30 redshifts should fall inside an interval of  $\Delta z=0.002$  is about 0.05. But what really makes such a peak interesting is how its properties are consistent with ideas concerning its origin. One cannot rely on purely statistical arguments because among these small redshifts random motions and cosmological recession tends to smear possible non Dopplerian peaks which thus must be only weakly discernible.

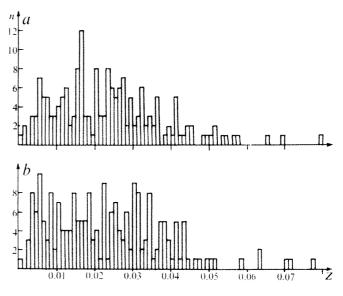


Fig. 2 Distribution of the redshifts of: a, s and sd objects combined: b, ds and d objects combined.

The value z=0.015-0.016 is half the value 0.03 and a quarter of 0.06. This might suggest a downward extension of Burbidge's relation<sup>3</sup>  $z=n\times0.061$ ,  $n=1, 2, 3, \ldots$ 

Figure 2a shows the redshift distribution for s and sd objects, and Fig. 2b for the classes ds and d. Evidently the peak originates mainly from the former ones, which is consistent with their more quasar-related nature.

If the peak is caused by intrinsic redshifts, the objects giving rise to it should be rather near, so that the cosmological redshift would not smooth it off. So it should originate chiefly from objects in the local supergalaxy. The dividing line on the celestial sphere given by Rubin et al. separates the low redshift ScI-galaxies (Region I) from the high redshift ones (Region II). Region II includes the central parts of the local supergalaxy. The shaded area in Fig. 1 represents the objects in and near Region I. Region II is chiefly responsible for the peak. Redshifts between z = 0.008 and z = 0.014 occur predominantly in Region I (ref. 7). From Fig. 1 it can be seen that at the peak and to the right of it the proportion of Region II redshifts increases.

Also in Arp's list<sup>17</sup> of interacting galaxy groups (five groups), six out of 16 hypothetical intrinsic redshifts (obtained by

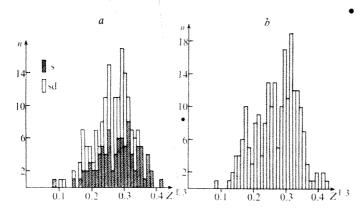


Fig. 3 Transformed  $(z \rightarrow z^{1/3})$  distribution of the redshifts of: a, s and sd objects; b, ds and d objects combined.

subtracting the redshifts of the primaries) would fall inside the peak z=0.015-0.016. Pecker et al. 18 have interpreted the redshift distribution of QSOs using the relation

$$z = aRT^3$$
,

where a is a constant, R the characteristic dimension of the QSO and T the temperature. The transformation  $z - z^{1/3}$  leads in the case of QSOs to a symmetric distribution around a plausible value of the temperature. It can be seen from Fig. 3 that the transformation  $z - z^{1/3}$  leads to a nearly symmetric distribution in the case of s and sd objects, but for ds and d objects the resulting distribution is less symmetrical. The small excess of numbers in the left hand side of the distribution of s and sd objects (Fig. 3a) comes from the peak at z = 0.005. This peak is seen also for the ds and d objects.

These data seem to support the view that compact and nearly compact Markaryan objects have anomalous redshifts. This is consistent with the general idea that anomalous redshifts are connected with compact objects.

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### Missing mass around galaxies: morphological evidence

RECENTLY we have obtained convincing empirical indications on the considerable role of hidden matter in the dynamics of single and double galaxies<sup>1</sup>. It seems that this matter is concentrated around massive galaxies, forming their coronas. The total mass of galaxies is about one order of magnitude greater than the mass of their visible parts.

Massive galaxies like our Galaxy or the Andromeda galaxy, M31, are surrounded by clusters of small companion galaxies. Searches for new effects of galactic coronas have led us to the discovery of a correlation between the morphological types of companion galaxies and their distances from the parent galaxies. It seems that elliptical companions are strongly concentrated around the parent galaxies whereas non-elliptical (spiral and irregular) ones populate preferentially the peripheral regions.

To derive this dependence we have collected the data on the companions of our Galaxy and of three external spiral galaxies —M31, M81 and M101 (refs 2-7). The adopted distances of these galaxies are as follows: 0.69 Mpc, 3.25 Mpc, and 7.25 Mpc (G. Tammann, private communication). The joint distribution (luminosity as function of distance from the parent galaxy) of the companions of all the four galaxies considered is given in Fig. 1. The regions populated by elliptical and non-elliptical companions are very sharply separated and the line of segregation is well defined.

To understand the nature of the regularity discovered we draw attention to the fact that the segregation of companion galaxies is connected essentially with one property—the presence or absence of the interstellar gas in the companion. Spiral and irregular companions, both of them containing much interstellar gas, are located to the right of the segregation line elliptical companions, containing practically no gas, are situated to the left of this line. A simple estimate shows that the segregation mechanism does not reduce to the tidal interaction which is too weak to produce such radical effects. If we reject the possibility of specific very artificial conditions during the formation of galaxies, there evidently remains the suspicion that the only agent capable of producing the interaction of the galaxies studied is intergalactic matter surrounding massive galaxies.

Let us suppose that the corona of a massive galaxy contains much gas. Consider a companion galaxy having its own gas

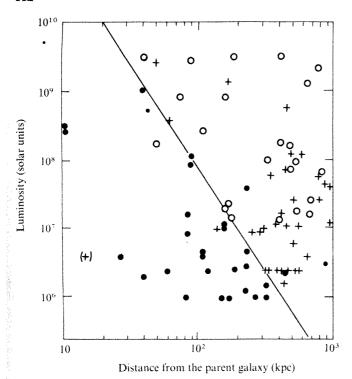


Fig. 1 The distribution (blue luminosity as function of distance from the parent galaxy) of the companions of our Galaxy and of three external galaxies: M31, M81 and M101. Elliptical companions populate the area to the left of the segregation line, non-elliptical companions to the right of this line. The presence of three spiral and irregular companions left of the segregation line is due to the projection effect; two elliptical companions right of the line have apparently more elongated orbits than other companion galaxies. •, Ellipticals; (), spirals; +, irregulars.

and moving through the coronal gas of the parent galaxy. The companion has a chance to preserve its gas only when the gravitational binding of this gas with the companion is stronger than the pressure of the coronal wind8. The gravitational binding energy can be expressed by the formula

$$U = -G\rho_g M_s R_s^{-1}, \tag{1}$$

where G is the gravitational constant,  $\rho_g$  the mean gas density of the companion galaxy,  $M_s$  and  $R_s$ —the mass and the characteristic radius of the companion. The expression for the pressure of the coronal wind is

$$p = \rho_c V^2, \tag{2}$$

where  $\rho_c$  is the density of the coronal gas near the perigalaxy of the companion orbit,  $V=(GM(R)R^{-1})^{1/2}$  is its orbital velocity and M(R) is the mass of the parent galaxy inside the companion orbit with the radius R. Since with an increasing distance R the density of the coronal gas decreases, the conditions for preserving the gas are the better the larger is R whereas in the inner regions of the corona we see only elliptical galaxies from which the gas is blown away by the coronal wind.

In order to estimate the mass of the gaseous corona,  $M_c$ , we have determined the density of the corona at various distances From the centre of the parent galaxy. This can be done, using the data on galaxies along the line of segregation, where the gravitational binding energy, |U|, is approximately equal to the pressure of the coronal wind, p.

According to Roberts9 the mass of dwarf irregular and spiral galaxies is proportional to their luminosity and the mean density of stars and gas is constant, and we have  $R_s \sim M_s^{1/3}$  and  $|U| \sim M_s^{3/3} \sim L_s^{2/3}$ . The segregation line between two morphological types of galaxies is a straight one  $L_s \sim R^{-3}$ , therefore  $|U| \sim R^{-2}$ . On the other hand, supposing a power law for the density of the corona

$$\rho_c \sim R^{-\alpha} \tag{3}$$

we obtain  $M(R) \sim R^{3-\alpha}$  and  $p \sim R^{2(1-\alpha)}$ . By comparing both expressions we get  $\alpha = 2$ .

The dependence  $\rho_c \sim R^{-2}$  leads us to the conclusion that outside the optically visible parts of the primary galaxy, where the density distribution is chiefly determined by the corona, the inner mass of the Galaxy,  $M(R) \sim R$  and the circular velocity  $V^2 \sim M(R)R^{-1}$  =const. This result agrees well with observational data on the circular velocity in the periphery of giant spiral galaxies10.

The derived density distribution agrees well with our previous results obtained from the dynamics of double galaxies1. For the distance  $R_0 = 15$  kpc we found for the coronal gas density  $\rho_0 = 2 \times 10^{-25}$  g cm<sup>-3</sup>. To estimate the total mass of the corona, the power law (3) cannot be integrated to infinity since the integral diverges. This shows that at large distances the density must decrease more rapidly than given by the law (3). On the other hand, we conclude from Fig. 1 that this law can be used until  $R^0 = 300$  kpc. Using this value of  $R^0$  as a limiting radius of the corona, we obtain for its mass

$$M_c \approx 4\pi \rho_0 R_0^2 R^0 \approx 2 \times 10^{12} \text{ solar masses}$$
 (4)

in good agreement with our dynamical estimate<sup>1</sup>. The mass found should be regarded as a lower limit of the mass of the corona since the adopted limiting radius Ro may be underestimated.

As noted by Oort11, the presence of large amounts of intergalactic gas is not surprising since the formation of galaxies is generally believed not to have been so efficient as to condense into stars all the diffuse matter in the Universe. Our data suggest that the intergalactic gas is not randomly distributed between galaxies, but is concentrated around massive galaxies, forming their coronas.

Dwarf elliptical companions of our Galaxy have been studied for stellar composition, which is almost identical to the oldest halo population stars<sup>4</sup>. This shows that star formation stopped soon after the formation of these galaxies. The space density of matter in the galaxies considered is small and under normal circumstances gas would be consumed at a very low rate12. A sudden cessation of star formation can be understood as an effect of the galactic corona, which blew the gas out from the nearby companions at an early stage of their history. But this points to the presence of the corona around the Galaxy just after its formation. At that early time the corona had to be gaseous.

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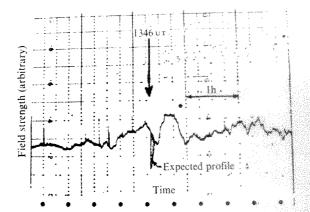
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#### An attempt to detect the effects of cosmic gamma-ray bursts in the lower ionosphere

DETECTION of gamma-ray bursts of cosmic origin by detectors onboard Vela, OGO, IMP-6 and OSO-7 satellites1-3 was one of the major surprises of observational astronomy recently. The bursts were observed over the energy range 7 keV to 1.5 MeV and were found to have time durations ranging from less than a second to about 80 s with integrated flux density between a few times  $10^{-6}$  and  $3 \times 10^{-4}$  erg cm<sup>-2</sup> for different events. From the 20 events that have been detected so far (from data accumulated over 5 yr) it seems that these events are distributed almost isotropically on the celestial sphere and occur at frequencies of about four to five each year, at the level of sensitivity of Vela satellites. In view of the absence of definitive positive associations with well known transient phenomena1,4 such as supernovae, galactic radio noise spikes, rapid atmospheric fluorescence increases, Cygnus X-3 radio flares or even with gravitational radiation events, continuous patrol for detecting these events through as many independent techniques as possible is very important to understand the nature of their origin.

So we have searched for the effects of these bursts on the VLF propagation characteristics in the D-region of the ionosphere; a technique<sup>5,6</sup> which earlier proved successful for the detection of X rays ( $\sim 1-10 \text{ keV}$ ) from sources such as Sco X-1. In short, the method consists of measuring the field strength of VLF propagation that shows absorption corresponding to the transit times of the X-ray emitting celestial bodies because of the resulting enhanced ionisation in the lower ionosphere. The effects of these bursts on the VLF propagation are investigated by examining the data on field strength variations recorded at Ahmedabad corresponding to 164 kHz transmission from Tashkent for several of the events recorded by the Vela satellites7. Further, since it is easier to delineate the ionospheric effects due to celestial photons in the absence of solar X rays, only those events that took place between the local times of 1900h and 0700h, corresponding to the geographical coordinates of the reflection point of these 164 kHz waves (32° N, 75° E) were chosen. Table 1 summarises the results of such a search for a number of bursts selected on the above criterion. The column on the observability of the event indicates whether the direction of the sky corresponding to the particular burst was within the visibility of the reflection point. Careful search of the recorded field strength data revealed no observable field strength variations associated with any of the above burst events. Figure 1 shows



Photograph of a typical record of the 164 kHz field strength variation recorded at Ahmedabad during March 28, 1972 event. This event had a total duration of ~ 65 s.

the relevant portion of a typical field strength record registered at Ahmedabad corresponding to the time of the occurence of the event dated March 28, 1971. The dotted line in the figure indicates the expected time profile7 of VLF field strength, if the X-ray burst event under consideration had an observable effect on the lower D-region of the ionosphere. The absence of an observable change in the field strength associated with the burst suggests that the X rays from this event did not have any significant influence on the ionisation of the D-region. Assuming that at least half of the uncertain cases should have been favourable from the observational standpoint, we conclude the results of the search for ionospheric effects of these events as negative.

Next, we estimated the electron production rates in the Dregion for a typical event such as of May 14, 1972, for which detailed spectral data8 are available above 7 keV. Since the atmospheric region between 90 and 70 km is most responsive to X rays between 1-10 keV (refs 6, 9), we have extrapolated the observed<sup>8</sup> power law number spectrum with an index of ~1.4 down to 1 keV for these calculations. Recent observations by Apollo 16 down to 2 keV justify such an extrapolation procedure (L. E. Peterson, private communication). The electron production rates for different heights are estimated for the two intensity peaks observed in the burst by the usual method using CIRA 1965 model of the atmosphere. The results are shown in Fig. 2 for the case of two zenith angles:  $\chi = 0^{\circ}$  and  $40^{\circ}$  together with that of Sco X-1 at  $\chi = 45^{\circ}$  for comparison Even though the electron production rates at the time of the second peak of the burst seem to be comparable with those from Sco X-1, the average production integrated over the duration of the burst of about 50 s is found to be at least a factor of two lower. One striking feature of the production profile is that the altitude at which the maximum rate of ionisation occurs for the burst is around 70 km compared with 85 km for Sco X4. At

					Та	ble 1 I	Burst s	earch d	lata					
Vela event	Date	l	ЭT	Duration (s)	3.5	on coo S/C co tion 1	ncide		Circle S/C	coinci	Radius	Estimated flux (erg cm <sup>2</sup> )	bility of the event	Whether effect is seen
number		h	min		α	δ	α	δ	α	δ	(degrees)	r	from the effection poin	ıt
6 9-4	17.10.1969	21	42	1.5					156	1	83	$4 \times 10^{-5}$	Uncertain	No
70-2	22.8.1970	16	50	10	143	65	205	25		Name of Part of Street, Texas	***************************************	$1 \times 10^{-4}$	Not visible	No
70-3	1.12.1971	20	01						91	30	16	$4 \times 10^{-5}$	Visible	No
71-1	2.1.1971	19	11	10	Poor i	ntersa	tellite	timings	š			$1 \times 10^{-4}$	Uncertain	No
71-3	18.3.1971	15	28		75	5	100	$-60^{\circ}$				$1 \times 10^{-4}$	Uncertain	No
72-1	17.1.1972	17	39	30 30	104	+9	136	29				$7 \times 10^{-5}$	Visible	No
72-2	12.3.1972	15	53		277	+1	298	+35				$5 \times 10^{-5}$	Not visible	No
72-3	28.3.1972	13	46						283	22	83	$1 \times 10^{-4}$	Uncertain	No
72-5	1.11.1972	18	57		11	$\pm 19$	309	56				$7 \times 10^{-6}$	Uncertain	No
73-2	10.6.1973	.0	ν,	and constant	••				25	-36	61	$1 \times 10^{-4}$	Uncertain	No

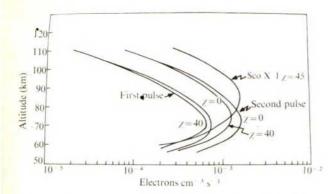


Fig. 2 Electron production rates as a function of altitude for the May 14, 1972, event. Also shown is the production rate due to the X rays from Sco X-1 corresponding to the zenith angle of 45°.

important consequence of such a profile is that the peak electron production rate for these types of bursts yields only low equilibrium electron densities because of the high values of recombination coefficients at the lower altitudes. The value of the recombination coefficient at 70 km is orders of magnitude higher than that at 85 km where X rays (~ 1-10 keV) from Sco X-1 have maximum influence and where consequently much higher equilibrium electron densities could be produced. Our estimates show that the electron density enhancement arising from this burst is insignificant at 70 km and is much less than 5% over the ambient value at 85 km. Because the burst under consideration is one of the strongest recorded so far, it is clear that the effects to be expected from the events listed in Table 1 should be many factors lower. We therefore conclude that for the type of bursts detected so far, the ionospheric effects are too weak to cause observable perturbations in the VLF propagation.

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#### No evidence for glacial origin of late Precambrian tilloids in Angola

KRÖNER and Correia1 claim that the mixtites in the Lower and Upper Tilloid Formations of the West Congo Sequence are "true tillites", although detailed study supports the idea that they originated as tilloids deposited by submarine mudflows in a geosyncline2. I question the validity of their arguments.

Mixtites which enclose striated clasts are not ipso facto tillites. Non-glacially striated stones occur in deposits unrelated to glaciation, and glaciclastic detritus may occur in non-glacial deposits. Clasts which have been abraded by nonglacial processes (including tectonism) can resemble glaciated clasts3.4. "Striated pebbles in mudflow deposits . . . have often been noted . . . and their similarity to glacially striated pebbles mentioned"<sup>4</sup>. If Kröner and Correia possess a diagnostic criterion which can provide an objective distinction between glacial and nonglacial striations on boulders, they would do well to state it. If their striated clasts are glacial-even today glaciers exist not far from the sea-that is still not evidence that the mixtites were deposited glacially, as till. (The West Congo geosyncline was at low to equatorial palaeolatitudes during tilloid accumulation5.) Glaciated stones in a delta would indicate glaciers in the hinterland but not the glacial deposition of delta sediments or the turbidites issuing from them. The striated boulders occur in a small area2. That restriction, which is unlike the situation for Pleistocene and older glacials, argues by itself against the extensive glaciation of land areas. To the north is a vastly larger area which lacks striated stones. Wagner and Wilhelm<sup>6</sup> examined over a thousand tilloid stones without discovering a single striation. The striated clasts occur near the south-eastern basin margin in Angola; the elevation of the source area may have been sufficient to cause local highland glaciation3.7.

The so-called "dropstones" are no such thing and have nothing to do with deposition of the tilloid formations; they are older. It is irrelevant to claim that a formation is glacigene because another, underlying, completely unrelated formation contains some small intraformational pebbles (which are not even erratics). Kröner and Correia found these clasts not in any tilloid formation but in the S3de formation (shales, siltstones, quartzites, carbonates and cherts) belonging to the Sansikwa Group. The Sansikwa and Lower Tilloid Formations are separated sharply by a disconformity/unconformity caused

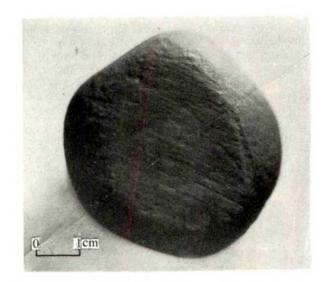


Fig. 1 Small, striated and faceted clast from the lower mixtite (M0), found west of Samba Cajú. The clast is also faceted on the back and these striations there have a markedly different direction from the front face.

by an erosional phase, and the tilloids carry eroded Sansikwa detritus as well as crystalline erratics. No dropstones appear in either the Lower or the Upper Tilloid Formation. The former encloses a thick mudstone level<sup>2</sup> which, if this formation were glacigene, could not fail to exhibit ice-rafted erratics. Kröner and Correia's Fig. 2 shows a minute pebble of intraformational siltstone in a subvertical position, only 8 mm long yet strongly deflecting silty laminae both underneath and above it: this is a compaction structure. A silt flake weighing only 0.25 g could not, dropping through water, have bored into bottom silt for a quarter of its length. Nor could it have been implanted subvertically: "in falling through even a few inches of water, pebbles rotate to place their maximum projection area (which is parallel to the long axes) approximately normal to the direction of fall"8. True dropstones, when small, lie parallel to the host rock lamination9, and are erratics. Nor is the pictured rock varve-like: it is similar to siltstone-shale alternations, such as the Tar Springs Sandstone, in which small silty pods also deflect and penetrate adjacent laminations (ref. 10, Plates 17C and 18C). Such current-transported intraclasts should not be confused with dropstones.

Graded bedding in tilloids and associated sandstones is frequent (though unknown from true tills or aquatills). Mudflows do enclose sandy lenses4. Internal stratification is rare, and not common, in tills; Boulton 11,12 has referred to flow tills which are mudflows.

The argument that turbidity currents could not have deposited the tilloids because they cannot carry coarse material, and that, consequently, these rocks must be glacial, makes no sense because this has never been claimed. Only mudflows have been envisaged, and their carrying capacity is well known2,3,

The tilloid formations are well stratified (the Lower Tilloid contains hundreds of tilloid beds). Their thickness varies from 0-500 m for the Lower Tilloid, and from 5 cm-200 m for the Upper Tilloid. Their greatest lateral extent in Angola is 100 km. Strong facies changes indicate lateral supply, with longitudinal input from geanticlines crossing the basin. That, and the absence of ice-rafting, the erosional bases which cut down 500 m into older strata and other evidence2, all argue against glaciomarine deposition.

Tilloid sedimentation was relatively deep marine, evidenced by widespread graded bedding (of normal type, not compositional layering) in tilloids and interbeds, and by the absence of shallow-water structures2,3. The tilloid formations are overlain by hundreds of metres of non-pebbly turbidites, and such thick, extensive, graded sequences do not occur on shelves. They pass up into shallow marine sediments with shallow-water structures. The laminated dolomite caps<sup>3</sup> which cover tilloids exhibit the characteristics of deep-water carbonates13, lacking shallowwater or lagoonal features. Quartz feldspar sandstone intercalations (with grading) occur in the tilloid formations, as well as among graded greywackes and mudstones in other formations, including flysch. Deep sea investigations show that clean sandstones can occur in a turbidite environment, and they have been reported from flysch troughs14-16.

The association of late Precambrian mixtites with limestone and dolomite is ironclad evidence against a glacial origin3. Experimental investigations and present day occurrences indicate that dolomite precipitation requires a minimum temperature of over 20° C. Recent and ancient precipitated carbonates and evaporites are concentrated at low latitudes<sup>17</sup>, where there are warm conditions. The absence of carbonate precipitates among Pleistocene, Permo-Carboniferous, Ordovician and Huronian glacials, confirms this.

Kröner and Correia's objections to nonglacial tilloid deposition are insubstantial; they did not dispute the main lines of evidence based on sedimentology and facies analysis2. The mudflow theory, which according to Kröner and Correia was "never accepted by any serious student", emerged from the detailed mapping of 77,000 km<sup>2</sup> of north-western Angola, covering 40% of the length of the West Congo geosyncline.

The results are available as map sheets with descriptive memoirs published by the Geological and Mining Service of Angola. No other late Precambrian mixtites in Africa, or indeed in the Southern Hemisphere, are as well documented. Serious and unserious students alike are free to interpret the assembled evidence as they will, for much remains to be learnt, but they should consider the whole of the evidence, not disregard essential parts of it.

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DR KRÖNER REPLIES: Schermerhorn concludes that our evidence in favour of a glacial origin for the Angolan mixtites1 is insubstantial and unsound. During a field excursion in 1973 he failed to convince any of the participants of a mudflow origin for his tilloids. He also rejected a glacial origin for the late Precambrian Numees mixtite in South West Africa2-6. Here I shall correct some of the misinformation he presents.

We have not claimed that mixtites which enclose striated clasts are ipso facto tillites, but that the numerous scratched stones in the West Congo 'tilloids' showing striations in various directions, sometimes crossing each other, are additional evidence for glaciogenic deposition of the host rock. Nobody has yet proposed a mechanism by which a small clast (5 cm diameter) can first be faceted and then striated in different directions (Fig. 1) during mudflow deposition, and I believe I have the wide support of students of glaciogenic deposits in identifying such striae as glacial.

Striated clasts are not restricted to a small area but have been found both in Angola and in Zaire1. It is not surprising that none has yet been reported from northern Angola since outcrops are extremely poor there.

There is no evidence to suggest a low to equatorial palaeolatitude during mixtite deposition in the West Congo basin. neither the precise age of these rocks is known nor has their palaeomagnetism been investigated. Detailed work on the Numees mixtite (a possible equivalent of the lower Wes Congo mixtite) indicates, however, a high palaeolatitude?

Our small dropstone, which is only one of several found weighs 11.4 g and not 0.25 g. Schermerhorn's claim that pebble drop through water with their flat sides normal to the direction of fall is incorrect and disproved by countless examples of dropstones piercing with their sharp edges or corners through the underlying lamination<sup>3-5</sup>. Even small dropstones penetrate the host rock lamination in this fashion; that has been demonstrated to Schermerhorn on a visit to the Numees Formation.

Schermerhorn and Stanton<sup>8</sup> state that "deposition of the tilloids was thus a type of geosynclinal turbidite sedimentation" and "... a subaqueous mudflow ... even becomes a turbidity current". Also "The beds of quartzite (intercalated in mixtite-A.K.) must have arrived as turbidity currents".

The mixtites extend for more than 800 km in the West Congo Basin and no mechanism can explain this distribution satisfactorily by mudflow deposition.

The association of mixtites with carbonates is characteristic of most late Precambrian glaciogenic deposits9 but, contrary to Schermerhorn's belief, it is not evidence against glacial deposition. The Numees tillite3-6 and the Bthaat Ergil Group in, Mauretania<sup>10</sup>, to cite only two well documented examples are both undisputably related to ancient glaciation but, nevertheless, contain carbonate beds.

Dr Schermerhorn claims boldly but incorrectly that the Angolan mixtites are the best documented of all such sediments in Africa or in the Southern Hemisphere, though only one publication had appeared about them before ours. If it were true, the argument about their origin would long have been settled in favour of glacial deposition.

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#### Work of fracture of natural cellulose

THE work of fracture (that is, the energy needed in order to form a fracture surface roughly at right angles to the grain direction) is, for timber, exceptionally high in relation to other mechanical properties and density. It compares well, on a weight basis, with that of ductile metals. Our tests on air dry (12% moisture content) pitchpine (Pseudotsuga taxifolia) for instance, give a mean value of  $0.92 \times 10^4$  J m<sup>-2</sup>. That is far higher than the energy needed to break the interatomic bonds in any given cross section of timber (which can hardly exceed 2 J m<sup>-2</sup>). It also seems to be noticeably higher than the energy that is likely to be absorbed by the fibre pull-out mechanisms which operate in conventional, artificial fibre composites<sup>1</sup>. There is reason therefore to suspect the existence of a special energy absorbing mechanism during fracture in trees and presumably, in other, plants.

Air dry timber has normally a macroscopic breaking strain in tension along the grain of about 1.0%, which is not very different from that of the cellulose fibrillae of which it is principally composed. Such a low breaking strain is not obviously compatible with the absorption of so much energy at fracture.

Cowdrey and Preston<sup>2</sup> have shown, however, that in the cell walls of timber the cellulose fibrillae are disposed in a preponderantly helical manner, making an angle which may vary between 6° and 30° in different cells but which always has the same sense in any one tree.

Page et al. have shown that when an individual cellulose cell is detached from its surroundings and pulled in tension, the walls buckle into the lumen in such a way that total longitudinal extensions of the cell of between 15% and 20% are possible. Page, however, was concerned with paper fibres and not with the fracture energy of timber.

We have made large ( $\sim 2$  mm diameter) model cellulose fibres by winding glass and carbon fibres into hollow helices with resin. When tested individually in tension, such tubes behave elastically up to a well defined yield point. Beyond that point the tube wall buckles and the equivalent of plastic yield takes place, enabling the tube to extend irreversibly by 10-20%, and thus to absorb a great deal of energy. We have made composite models resembling timber, by glueing together a number of parallel tubes of this type, and these exhibit experimental works of fracture up to about 40 × 104 J m<sup>-2</sup>, which may be higher than any value previously recorded for a non-metallic material.

We have observed that the fracture behaviour of timber, watched under the optical microscope, much resembles that of our fibre-composite models. The first irreversible event to be observed is the lateral separation of many of the cells in the immediate neighbourhood of the fracture (see ref. 4). That enables the walls of the cells in that region to buckle, and the cells themselves to elongate. Thus, although the elastic strain in the timber as a whole seldom much exceeds 1.0%, the cells close to the fracture surface typically extend 15-20% before breaking, absorbing much energy as they do so.

The adoption of a helical arrangement of reinforcing fibrillae naturally, involves some reduction in the maximum attainable value of longitudinal Young's Modulus. By calculation this reduction seems to be proportional to the square of the cosine of the helical fibre angle. That is equivalent to the loss of about 1% for a 6° helix, increasing to 25% for a 30° helix. Such a loss of stiffness may well be regarded as an acceptable price to pay for the acquisition of so much fracture toughness.

We acknowledge support from the Science Research Council for this research. A patent application is pending.

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#### Imparting strength and toughness to brittle composites

HIGH strength and high toughness are usually mutually exclusive in composites which have brittle filaments in a brittle matrix. The high tensile strength characteristic of strong interfacial filament-matrix bonding can, however, be combined with the high fracture toughness of weak interfacial bonding if the filaments are arranged to have alternate sections of high and low shear stress (and low and high toughness). Such weak and strong areas can be achieved by appropriate intermittent

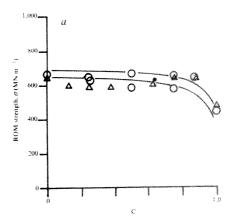
coating of the fibres. The strong regions ensure that the filament strength is picked up. Randomly positioned weak areas effectively blunt running cracks by the Cook–Gordon mechanism<sup>1</sup>, which in turn produces long pull-out lengths with an associated large contribution to toughness. Boron–epoxy composites of volume fraction 0.20–0.25 have been made in this way; they have fracture toughnesses of over 200 kJ m<sup>-2</sup>, and they retain rule of mixtures tensile strengths ( $\sim$ 650 MN m<sup>-2</sup>). At the volume fractions used, that apparently represents  $K_{\rm IC}$  values greater than 100 MN m<sup>-3/2</sup>.

When the interfacial bond between fibre and matrix is strong in composites which have brittle filaments and a brittle matrix, fracture is often caused by rapid matrix cracks which break through all filaments in their paths. The toughness of such composites is low because, in general, the critical transfer length associated with strong interfacial bonding is small, which limits the various components of the total toughness—the 'surfaces' component, Piggott-Fitz-Randolph stress redistribution and Cottrell-Kelly pull-out (see, for example, ref. 2). The critical length is given by  $l_{\rm crit} = \sigma_{\rm f} d/2\tau$ , where  $\sigma_{\rm f}$  is the filament strength, d the filament diameter, and  $\tau$  the interfacial shear strength.

A general increase of  $l_{\rm crit}$  by lowering the filament-matrix shear bond will increase the toughness, and a relationship between rule of mixtures (ROM) strength,  $\sigma$ , and total composite toughness, R, may be developed by recognising that in, general terms,  $R \propto l/\tau$ . Weak interfaces throughout the composite, however, reduce the tensile strength quite significantly. The question that presents itself is whether there are means by which the ROM tensile strength can be maintained along with high toughness values.

Marston<sup>3</sup> suggested that, providing there were 'sufficient' regions of high interfacial shear stress to ensure that the ROM strength was picked up, the rest of the composite could have quite weak interfacial bonds. Were such a composite to be laid up randomly with respect to weak and strong regions, both high strength and high toughness should be produced simultaneously. Then, if the lengths of the strongly bonded regions were greater than the critical length associated with the strong interfacial τ, the filament strength should be attained. At the same time, those weak interfaces situated randomly ahead of running cracks would serve to blunt the cracks by debonding.

Weakly and strongly bonded interfaces can be achieved by coating intermittently the filaments with some suitable substance before composite lay up. Experiments with silicon vacuum grease (SVG) and polyurethane varnish (PUV) coatings are described here; the uncoated regions have high interfacial shear stress and the coated regions are 'weak'. Tensile strength and toughness specimens were made from layers of intermittently bonded, epoxy composite tape, manufactured on a drum apparatus with a device for coating the filaments before lay up (A.G.A., unpublished). The tape (similar to Avco Rigidite, Prepreg tape) consisted of a 250 µm monolayer of B/W filaments in EPON 828 epoxy, backed, for ease of handling, on 760 mm wide nylon scrim cloth about 50 μm thick. The tensile strength specimens consisted of 2 layers of the tape, in 100 mm × 6 mm strips, with end tabs reinforced by additional layers. Most toughness measurements were made on flat sheet edge-crack specimens, similar to American Society for Testing and Materials 'compact tension' specimens in profile. These consisted of 10 layers of tape in panels  $76 \text{mm} \times 76 \text{mm}$ . To prevent the composite arms above the crack from shearing off under load, two outside layers of tape were arranged on each side of the specimens, with the filaments parallel to the crack. The central core of the specimen thus consisted of six unidirectional filaments perpendicular to the starter crack, where, within the limitations of the specimen and tape preparation method, the coated and uncoated layers occurred randomly, relative to each filament. Fracture toughness in the edge-crack specimens was measured for increments of crack area, using Gurney's sector area technique4.



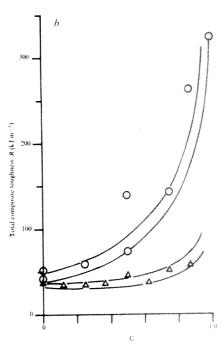


Fig. 1 Tensile strength (a) and edge-crack fracture toughness (b) plotted against the percentage coating, C.  $C = I_0/(l_c + I_{uc})$ ; where  $I_c$  is the coated length,  $I_{uc}$  the uncoated length, and  $(l_c + I_{uc})$  is the repeat distance of the coating pattern along the filaments.  $\bigcirc$ , Results with PUV coating;  $\triangle$ , results with SVG coating.

Tensile strength and edge-crack fracture toughness, for both SVG and PUV coatings, have been plotted against C, the 'percentage coating' (Fig. 1a, b). The tensile data remain at or about the ROM value until C becomes greater than 0.8. In the same range of C, the toughness with SVG coating, shows a modest increase over the initial (C=0) case. In contrast, the PUV coatings give marked improvements in toughness when the percentage of coating is increased (Fig. 1a, b): in the edge-crack specimens, with  $V_f=0.25$ , toughnesses of about  $100 \, \text{kJ m}^{-2}$  are produced for C=0.5, and values over 200 kJ m<sup>-2</sup> occur at large C.

Superimposed on the figures are the predictions of analyses for  $\sigma$  and R, details of which are to be presented elsewhere. Both coating materials maintain tensile strengths of the order of the ROM values up to large C, which suggests that both produce similar coated interfacial shear-strengths. Their effects on toughness are, however, different, which suggests that interfacial shear-strength may not be the controlling parameter for toughness. With PUV it seems that Cook-Gordon debonding takes place (average pull-out lengths of

 $l_{\rm crit}/4$  being augmented by additional debonding), but it is apparently absent in the case of SVG.

The Cook-Gordon mechanism itself seems to be only a small toughness sink, but the extra debond lengths markedly increase the pull-out contribution to toughness. The manner in which interfacial properties, other than shear strength, are affected by the coating procedure is therefore both interesting and important, because it is probably the 'toughness' of the interface that is of ultimate concern, rather than the 'strength'. Tensile debonding ahead of a crack, envisaged by Cook and Gordon, is Mode I fracture in the nomenclature of fracture mechanics; the shear debonding implicit in the Outwater–Murphy debonding analysis for toughness, is in the 'forward sliding' Mode II. Each mode has its own toughness, with explicit relationships with interfacial tensile and shear strengths, which are not obvious.

Further information on the intermittent bond concept is to be presented elsewhere.

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# Effect of temperature on recombination luminescence and electron tunnelling

RECOMBINATION luminescence which occurs in alkaline glass after  $\gamma$  irradiation at 77 K, decreases in intensity when the glass is cooled. Between 60 K and 4 K the activation energy is probably about zero. This seems to be direct proof of a tunnelling mechanism.

Electron stabilisation occurs in deep intermolecular traps (2–3 eV) during low-temperature radiolysis of polar glasses. After γ irradiation, at 77 K, of aqueous alkaline glasses containing efficient electron scavengers (NO<sub>2</sub>-, NO<sub>3</sub>-, Fe(CN)<sub>6</sub><sup>3-</sup>), a long range electron transfer occurred, apparently by a tunnelling mechanism<sup>1,2</sup>. The observation and kinetic study of prolonged isothermal luminescence (ITL) in glassy organic matrices<sup>3,4</sup> and in alkaline glass<sup>5</sup> at 4 K and 77 K, which results from the recombination of trapped electrons (e<sup>-</sup><sub>t</sub>) and positive ions, confirms the validity of this mechanism; it also shows that the phenomenon is general in glassy materials.

Our aim was to study the temperature effect on recombination kinetics, using the ITL technique in the range between 77 K and 4 K, where electron tunnelling is apparently the predominant mechanism.

Sodium hydroxide glass (10 mol dm<sup>-3</sup>) containing phenol (0.03–0.3 mol dm<sup>-3</sup>) was used because ITL intensity is increased by the presence of phenol<sup>3</sup>. Irradiations were carried out at 77 K with a cobalt-60 source at a dose rate of 5 krad min<sup>-1</sup> for periods of 5–15 min. After recording the ITL decay for about 10 min, liquid helium was transferred into the cryostat, whilst monitoring of the luminescence continued.

Following  $\gamma$  irradiation of NaOH glass, whether pure or with phenol, at 4 K or 77 K, ITL can be observed for up to several hours. The kinetics of the decay, just as in the

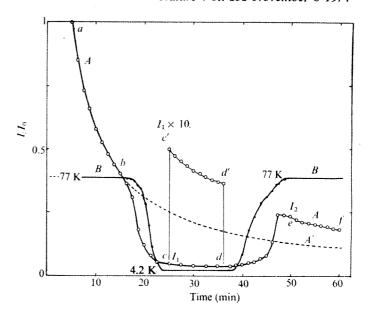


Fig. 1 Recombination luminescence in NaOH glass (10 mol dm<sup>-3</sup>) containing phenol (0.03 mol dm<sup>-3</sup>), irradiated with γ rays (dose: 50 krad) at 77 K. A, Luminescence intensity (relative units): sections ab and cd, ITL at 77 K and 4 K (c'd' enlarged 10 times); sections bc and de, luminescence intensity during cooling to 4 K and warm up to 77 K; section ef, ITL at 77 K after cooling and warm up cycle. A': extrapolation of the initial ITL decay at 77 K based on the linear plot of I/I₀ against time, according to equation (1). B, Temperatures corresponding to light intensities of A.

case of organic glasses<sup>4,6</sup>, can be described by the relationship

$$I_0/I = 1 + k(t - t_0) \tag{1}$$

in which  $I_0$  and I are ITL intensities at the beginning of the observation  $t_0$  and at time t; and k is a coefficient (with the dimension of a rate constant) depending on the duration of  $\gamma$  irradiation, but independent of dose and dose rate. Under identical experimental conditions, the kinetics observed at 4 K and 77 K coincide. The intensity of ITL after irradiation at 4 K is, however, approximately five times larger than after irradiation at 77 K.

Figure 1 shows the change of luminescence intensity with temperature in alkaline glass irradiated at 77 K. The decrease in temperature (Fig. 1, section bc) brings about a reduction of luminescence intensity by a factor of about five. Thus, the ITL intensity after irradiation at 4 K is actually about 25 times that after irradiation at 77 K and subsequent cooling to 4 K.

When the temperature is raised again (section de), the luminescence intensity increases and reaches a value higher than the normal ITL curve at 77 K (curve A'). The kinetics of ITL decay in the sections ab (77 K), cd (4 K) and ef (77 K) can all be represented linearly by equation (1). This indicates that the same recombination mechanism must be predominant under the experimental conditions of these luminescence decays.

This temperature effect is reproducible and can be repeated on the same luminescence decay curve, as long as there is sufficient light intensity.

Because the luminescence intensity at any moment is proportional to the reaction rate of recombination, it can be considered to be proportional to the rate constant when temperature is the only parameter which changes. Therefore, the fivefold decrease in luminescence intensity observed during cooling from 77 K to 4 K suggests an exceedingly low activation energy. In fact, there is practically no change between 60 K and 4 K.

From our thermoluminescence experiments (unpublished), the activation energy for charge recombination, ealculated by the initial rise method for the glow peak at 92 K, was found to be  $\approx$  7 kcalorie mol<sup>-1</sup> ( $\approx$  0.3 eV). This is an activation energy for a thermal detrapping process which is predominant at the glow peak. Its contribution decreases with decreasing temperature, and therefore the apparent activation energy calculated from the Arrhenius equation gradually decreases until it is practically zero below 60 K, when tunnelling seems to be the only process. Zero activation energy seems to be direct proof of a tunnelling mechanism in the decay of trapped electrons at sufficiently low temperatures.

These results confirm the general rules concerning the contribution of tunnelling to the rate constant of a chemical reaction\*. They show that below a certain temperature tunnelling plays the predominant role.

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#### Volatilisation of terpenes from Salvia mellifera

WE have found that branches of Salvia mellifera, an aromatic shrub that grows in coastal southern California, produce sufficient quantities of terpenes to make a significant contribution to the atmospheric content of organic compounds. Our simultaneous qualitative and quantitative analyses of compounds volatilised by intact plants in controlled conditions represent an advance on previous measurements of organic compounds in the atmosphere surrounding plants1-3.

Salvia mellifera, the California black sage, is a shrub (1-2 m tall) distributed throughout the coastal regions of California from the San Francisco Bay area southward. In some immediate coastal regions it is the dominant plant species. Its herbaceous leaves, which are covered with glandular, terpene-containing trichomes, usually drop by the end of the long summer drought.

Net photosynthesis (under saturating light) and dark respiration were measured in an intact branch of a potted plant using a gas analysis system like that previously described4. The sample was contained within a cuvette where temperature and humidity were controlled. Photosynthetic and respiration rates were determined for various temperatures (Fig. 1).

The volatile constituents of the air emerging from the gas exchange cuvette were trapped in stainless steel loops which were packed with Pyrex wool and immersed in liquid nitrogen.

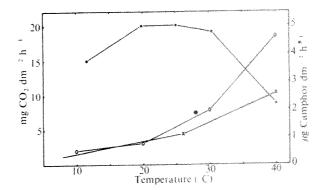


Fig. 1 Temperature curves for photosynthesis under saturating light (●), dark respiration (×) and camphor volatilisation (○ in S. mellifera.

A cryogenic pump maintained flow through the loops (1 I mine for 10 or 20 min). The organic volatiles were separated from the water which accompanied them and analysed qualitatively by gas chromatography-mass spectroscopy5. Gas chromatography alone was used for quantitative analyses. For convenience all quantitative measurements were based on camphor volatilised. The only other major volatile component was 1,8-cineol (23%), levels of which correlated closely with those of camphor (50%). Other terpene hydrocarbons (a-pinene, 8%; β-pinene, 4%; camphene, 4%; limonene, 3%; cymene, 2%; myrcene, 2%; β-phellandrene, 2% and γ-terpinene, 2%), were difficult to quantitate and were not measured.

Steady-state rates of terpene (camphor) volatilisation in the light and dark were determined by placing the plant in the chamber at constant temperature and humidity and monitoring the camphor produced until the rate of volatilisation was constant. Rates were directly proportional to leaf temperature (Fig. 1), and were the same in both light and dark.

Daily carbon gain and terpene loss by S. mellifera can be calculated using the temperature curve for the steady-state rate of terpene volatilisation and the gas exchange characteristics (Fig. 1) and the natural daily temperature regime of an area where the plant is dominant<sup>6</sup>. Figure 2 gives the temperature regime and calculated levels of terpene loss and gas exchange for April 1972 at a coastal site (Camp Pendleton, California) where S. mellifera is dominant. During this period the plant was physiologically active. The calculated rate of terpene volatilisation was 13.3  $\mu g\ dm^{-2}\ d^{-1}$ .

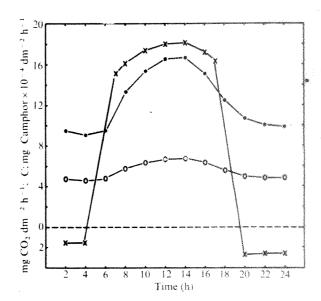


Fig. 2 Mean hourly temperatures for April 1972 at Camp Pendleton ( ) and calculated rates of CO2 exchange assuming saturating light during the light period (x) and camphor volatilisation (O) for S. mellifera at that site.

If 5.2 g of CO<sub>2</sub> is required to produce 1 g of camphor (18 mol CO<sub>1</sub>→6 mol 3-PGA→6 mol acetyl-CoA→2 mol mevalonic acide 1 mol camphor), the loss of photosynthate through camphor volatilisation was 0.03% in April. Since camphor represents 50% of the terpene pool in S. mellifera, the total loss of carbon by terpene volatilisation was 0.06% during April. This value is about two orders of magnitude below Went's predicted 5% loss of photosynthate as volatile terpenes from Artemisia tridentata1.

S. mellifera comprises 45.7% of the vegetation cover at the Camp Pendleton site. Based on the rate of volatilisation for April and a leaf area index of 2.6, 3.1 kg terpene km<sup>-2</sup> d-1 would have been released into the atmosphere from this site in April. This rate is one order of magnitude below that predicted for A. tridentata (50 kg km<sup>-2</sup> d<sup>-1</sup>) (ref. 1).

The measured rates of terpene volatilisation from intact branches of S. mellifera, which is highly aromatic, do not account for the high levels of atmospheric organics measured by Rasmussen and Went2 in the field. Their observations that levels increased immediately after fields were mowed and at times of maximum leaf drop in deciduous forests, but not with the appearance of new foliage in the spring, suggest that the source of volatiles was primarily decomposition or loss from dead cells, rather than volatilisation from intact plant material. Detailed analyses of field samples are required to determine the nature of volatile organics in the atmosphere; contrary to general assumption, many may not be terpenoid2.

Our study demonstrates significant terpene volatilisation from healthy, intact leaf material of S. mellifera. This loss of carbon represents a substantial metabolic cost which implies that it confers some benefit on the plant. Terpenes, especially camphor and 1,8-cineol, from S. mellifera have been suggested to act as phytotoxins in the ecosystem7. Quantitative data such as ours will allow a more comprehensive analysis of this proposal by indicating the contribution of Salvia populations to natural levels of terpenes in the ecosystem-levels at which the compounds must be active.

The terpene content of the leaves, which was measured as 31.5 mg dm<sup>-2</sup> by steam distillation, represents a large amount of potentially volatile material, which may lead to intervals of high atmospheric terpene levels at the time of leaf senescence. This might be especially important in the coastal sage communities of California, where leaf drop and high summer temperatures occur simultaneously.

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Lead levels in the water of suburban Glasgow

THE association of soft water with increased plumbosolvency from domestic cold water systems1 has been shown by Beattie et al.2 to result in elevated levels of blood lead in persons resident in lead plumbed houses in soft water areas. This effect was particularly marked in people resident in houses supplied with cold water from lead lined storage tanks. Here we report a study carried out in an area of Glasgow whose residents were principally owner-occupiers of social classes one and two.

The houses studied were identified from the intersections of a grid placed over a 1 in 2,500 scale Ordnance Survey map. Each house was numbered and selected from a table of random numbers. This gave a total of 120 residences. After selection, householders were invited to take part in this survey of the lead status of their homes. The person first contacted was usually the person studied from each household. No person was included in the survey who had been resident in the house for less than 1 yr. Each participant had 10 ml venous blood withdrawn, heparinised and stored in ice. At the same time 250 ml bottles were left for a collection of the first morning flush of water from the cold water system.

Erythrocyte  $\delta$ -aminolaevulinic acid dehydratase (EC 4, 2, 1, 24) (ALA.D) the second enzyme of the haem biosynthetic pathway, which is often taken as a bioanalytical measure of lead exposure, was measured by the Commission of the European Communities Standard Method<sup>3</sup>. The results were expressed as µmol ALA utilised per ml RBC per min (Units). Blood and water lead levels were measured by flameless atomic absorption spectrophotometry (Table 1).

In the assessment of the results, the houses were arbitrarily divided into two groups; those less than 20 yr old (group A) and those greater than 20 yr old (group B). This division was chosen because before 1939 these houses were constructed with lead plumbing, and until 1967 lead 'link' pipes were used to connect the domestic copper system to the cast iron main. There were 50 houses in the survey, 12 in group A and 38 in group B. On the basis of this division, it was found that there was a highly significant difference in the water lead content of the two groups. The older group of houses (group B) had a mean water lead content of about 350 µg l<sup>-1</sup>, that is, ten times greater than the mean of 35  $\mu$ g l<sup>-1</sup> in group A. Indeed, in the latter group no water lead value was greater than 100 µg 1-1 (WHO upper acceptable limit), and 82% of the houses in group B were above this limit. Associated with this increase in water lead levels there was a significant 1.5-fold elevation of blood lead in the occupants of the older houses. There were, however, no significant differences in the age of resident examined, the duration of residence or of the sex ratio between the groups. When the water lead (PbW) and blood lead (PbB) levels were compared for all subjects in the study, a significant linear regression was found with regression coefficient r = 0.417 using the equation PbB = 0.018PbW+22.9.

Table 1 Lead in Glasgow water				
	Group A (< 20 yr)	Group B (> 20 yr)	Significance	
No. in group	12	38	Significance	
Mean age of house	13.5 ± 4.4	$42.6 \pm 4.2$	and prompty page.	
Time of residence	$8.8 \pm 5.3$	16.1 + 12.5	NS*	
Age of resident	$41.3 \pm 18.8$	$46.7 \pm 17.8$	NS	
Sex ratio (female:male)	2:1	29:9	produce.	
Water lead (µg 1 <sup>-1</sup> )	$34.6 \pm 29.5$	$353.1 \pm 255.6$	P < 0.001	
Blood lead (µg per 100 ml)	19.8± 8.4	$30.5 \pm 10.6$	P < 0.01	
δ-aminolaevulinic acid dehydratase (units)	27.8±13.2	22.3 ± 10.4	NS	
ALA.D Residence > 5 yr	20.4 - 12.0 (10)	10.0 : 5.1/20)	D . O 002	
(No. of subjects)	30.4 ± 12.9 (10)	18.9 ± 5.1 (30)	P<0.005	

<sup>\*</sup> Not significant

In the initial analysis, no significant difference was found between the groups for ALA.D activity. When subjects who had been resident in the houses for less than 5 yr were removed from the analysis, however, resulting in 10 subjects in group A houses and 30 in group B houses, a highly significant difference in ALA.D activities was observed. The difference was of the same order as the difference in blood lead values (1.5 times). As has been often previously shown, there was a highly significant negative exponential regression relationship between the blood lead and blood ALA.D with r = -0.46 using the equation ALA.D =  $34.5e^{-0.017 PbB}$ 

Of perhaps greater interest was the observation that for all residents in the survey there was a significant negative exponental regression relationship between erythrocyte ALA.D activities and water lead levels, using the equation ALA.D (=) 25.7e -0.0006 PbW

These results clearly demonstrate the possible environmental hazard from lead plumbing, especially in a soft water area such as Glasgow. It is difficult, indeed impossible, to determine the point at which low levels of lead ingestion may harm exposed persons. It is, however, significant that biochemical changes can be observed in ALA.D at blood lead levels as low as those found in this survey which represents the normal levels found in a large urban and suburban population. Remedial measures should, and indeed are, being taken in such areas of possible hazard, including the removal of lead plumbing and the 'hardening' of water with calcium and magnesium salts to lower the plumbosolvency of the water.

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#### Feature processing in the perception and production of speech

It has long been argued that the perception and production of human speech are mediated by a common mechanism<sup>1-3</sup>; nonetheless, convincing evidence has not yet been obtained in favour of this view4. We discuss here, however, a series of experiments in perceptuo-motor adaptation that provide fairly direct evidence favouring the existence of such a mechanism. Our findings indicate that this mechanism processes information about individual phonetic features of consonants<sup>5-7</sup>, rather than processing these consonants as integral units.

Subjects were instructed to utter a given consonant + vowel syllable immediately after listening to repetitions of a selected adapting syllable. The utterances were analysed to determine whether a feature of the waveforms systematically varied as a function of perceptual adaptation. We concentrated on a single phonetic feature-voicing, in word-initial stop consonants. In English, voicing minimally distinguishes voiced from voiceless stop consonants-[b] from [p], [d] from [t], and [g] from [k]. This feature was chosen because: (a) it has an acoustic correlate that can be readily measured from real speech waveforms, (b) numerous data on perceptual adaptation have been obtained for this feature8-11, and (c) the feature serves to mark phonemic distinctions in virtually all natural languages12.

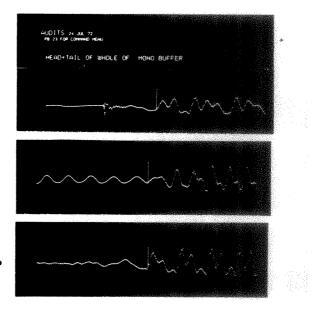


Fig. 1 Sample oscillographic displays of the utterances, illustrating the method used to analyse VOTs. The top display shows the initial segment of a [bi] utterance containing a short-lag VOT (voicing onset occurs shortly after the release burst of the consonant). The pointer T indicates the onset of the release burst, the vertical line to the right of T marks the onset of voicing, the time difference between the T pointer and the vertical line is displayed at the bottom right of the oscilloscope screen. For this utterance, the VOT is measured to be +11.6 ms. The middle display shows a segment of a prevoiced [bi] syllable (voicing onset precedes the release burst of the consonant). For prevoiced [bi]s, the pointer T (not shown in the display segment here) is set at the onset of voicing, while the vertical line cursor is aligned with the onset of the release burst. The time difference between the two markers is taken as the measure of VOT, in this case -86.6 ms (negative VOT values signify prevoicing). The bottom display shows a segment of aspiration and the onset of voicing for a [pi] utterance. The T pointer is set at the onset of the release burst (not shown in this display segment), and the vertical line cursor is moved to the position of voicing onset as in the case of the short-lag VOT [bi] utterances. The actual screen size is  $14 \times 13$  inches, and the vertical and horizontal display scales can be magnified to facilitate the marking procedure. The displays are taken from ref. 13

During speech production, voicing distinctions for word initial stop consonants can be signalled by voice onset time (VOT), a temporal relation between the release of oral closure and the onset of vocal cord vibration. Voiceless stops ([p] [t], and [k]) are normally produced with an onset of vocal convibration that lags behind the release of closure by more than 30 ms, whereas voiced stops ([b], [d], and [g]) are produced with an earlier onset of voicing. In this research the question of perceptuo-motor control was studied by analysing VOI values for a sample of approximately 5,000 consonant + vowe utterances, articulated under different conditions of perceptua adaptation.

Throughout the tests, three adapting syllables were used-[i], [pi], and [bi]. These syllables were five-formant synthetic speech patterns generated on a terminal analogue speech synthesiser (acoustic properties of these syllables are described elsewhere13). Subjects were tested individually in a sound insulated chamber. Forty adaptation trials were administered in blocks of 10 during an hour-long session, with a 5-min resi period between blocks. On each trial in a given block, the subjects were presented with 70 repetitions of a single adapting stimulus, with an inter-repetition interval of 350 ms. Subjects listened to the adapting syllable binaurally over headphones. They were instructed to hold their tongues firmly between their teeth and lips while listening and were told to minimise subvocalisation. After 70 repetitions of the adapting syllable were presented, subjects uttered a single CV syllable in a natural voice, as soon as possible (mean response latency < 1.5 s).

Each utterance was analysed oscillographically, aided by the AUDITS computer program <sup>14</sup>. A cursor was manipulated to mark the onset of the consonant release burst and the onset of voicing, and the time difference between the two marks was displayed on the oscilloscope screen to the nearest 100  $\mu$ s. The accuracy of each VOT measurement was estimated to be within  $\pm$  1 ms, except in the case of voiced consonants with VOTs near 0 ms, where the accuracy was reduced to about  $\pm$  3 ms. In all cases, accuracy was limited by our ability to specify the onset of voicing. Examples of the oscilloscope displays are shown in Fig. 1.

In the first experiments<sup>13</sup>, subjects were instructed to utter the syllable [pi] or [bi] on each block of trials after repetitive listening to [i], [pi], or [bi]. The results for the [pi] utterances showed a significant decline in VOT values after perceptual adaptation to [pi], compared with the VOT values obtained after adaptation to the isolated vowel [i] (P < 0.05). Thirteen of the 16 subjects showed this decline in VOT as a function of perceptual adaptation; the mean VOT shift was 5.6 ms. For the [bi] utterances, no systematic shift in VOT was obtained after perceptual adaptation to [bi], compared with VOTs obtained after adaptation to [i].

The asymmetry observed between the effects for [pi] and [bi] utterances was in accord with a number of additional facts. Whereas the distribution of the VOT values for the [pi] utterances was approximately Gaussian under all test conditions, and showed no highly elevated response peak, the distribution for the [bi] utterances was non-Gaussian and showed a very strong clustering of responses within the narrow range between 0 and 20 ms VOT. The presence of a well-defined target region for the VOT production of the [bi] utterances—a consequence of simple mechanical coupling by airflow<sup>15</sup>—may have accounted for their resistance to adaptation. Converging evidence of asymmetry between the processing of voiced and voiceless stops has been obtained in studies of perceptual adaptation<sup>8,9</sup>, speech production<sup>12,16,17</sup> and neonate perception<sup>18</sup>.

We were able to conclude that the perceptuo-motor adaptation effect for [pi] represented the fatiguing of a mechanism that mediates an aspect of both speech perception and production. In reaching this conclusion, we ruled out two alternative interpretations—perceptuo-motor compensation<sup>19</sup> and voluntary mimicry of the adapting syllable (see ref. 13 for details). Having demonstrated the existence of a perceptuo-motor effect, we conducted a second series of experiments to begin to determine the nature of the mechanism tapped by this procedure. We wanted to find out in particular whether the mechanism processes information about the stop consonants in toto or whether it

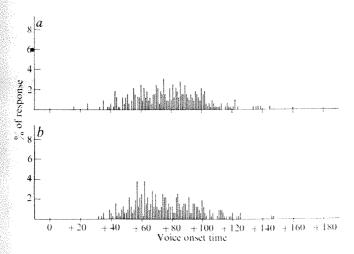


Fig. 2 Frequency distribution of the [ti] utterances for the group of 32 subjects after perceptual adaption to [i] (a) or [pi] (b). This display provides a good record of the range and distributional pattern of VOT values, although it does not easily reveal the small but significant shift in VOTs between the two test conditions.

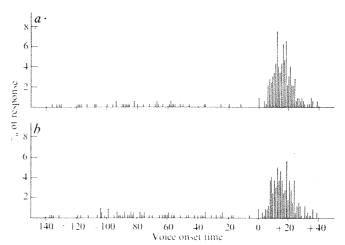


Fig. 3 Frequency distribution of the [di] utterances for the group of 32 subjects after perceptual adaption to [i] (a) a [bi] (b).

processes information about the individual phonetic features that comprise these consonants<sup>5-7</sup>.

In linguistic theory, the notion that consonants can be represented as bundles of distinctive features—features such as 'voicing' and 'place of articulation', to a first approximation—provides the chief means of accounting for relationships of phonetic similarity. Previous studies of perceptual adaptation<sup>8,9</sup> provided evidence that linguistic features may be processed during some stage of speech perception. The present aim was to determine whether these features are extracted at the level of processing which mediates both speech perception and production. We studied this question by examining the case in which both the adapting syllable and the selected utterance shared the relevant voicing property, but differed from each other in place of articulation.

In these experiments, subjects were presented the adapting stimuli [i], [pi] and [bi] as before. But now, the subjects' task was to utter the syllable [ti] or [di] after each group of 70 adapting repetitions. For each of the two CV utterance types, perceptual adaptation to [i] was counterbalanced across blocks of ten trials with perceptual adaptation to the CV syllable belonging to the same voicing category as the utterance.

The results of the experiments appear in Figs 2 and 3, where the VOT frequency distribution of the [ti] and [di] utterances are shown for each test condition. A total of 1,280 utterances was examined in these experiments (320 in each of the four experimental conditions). For [ti] utterances, a systematic decline in VOT was obtained after adaptation to [pi], compared with the VOT values obtained after adaptation with the isolated vowel (P < 0.01). Twenty-three of the 32 subjects showed this effect; the mean magnitude of effect was 3.2 ms. This average magnitude was about 2% larger than that obtained for [pi] utterances with the same subjects (based on data presented in ref. 13 and additional tests). The obtained effect for the voiceless stops thus seems to be entirely featurespecific. No systematic shift was obtained in the VOT values of the [di] utterances after adaptation to [bi]. The asymmetry observed between the effects for [ti] and [di] utterances corresponded to the asymmetry obtained earlier for [pi] and [bi] utterances. In both cases, perceptuo-motor effects were found for the voiceless stop consonants but not for their voiced counterparts. The corresponding difference in the distributional patterns for VOT held as well for the present data (see Figs 2 and 3).

The results for the [ti] utterances indicate that the stage of processing subserving both the perceptual and motor systems of speech performs at least one highly specialised function—namely, processing information about the voicing property of the consonant. Since the [pi] adapting syllable contained a consonant with a different place of articulation from the [ti] utterance, the perceptuo-motor effect cannot be attributed to a mechanism that processes consonants as indivisible units.

With the present experimental technique, in combination with direct study of certain laryngeal factors 20-22, we hope to further define properties of this perceptuo-motor component of the speech system.

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#### Recognition of mother's voice in early infancy

CERTAIN characteristics of the human voice at normal levels have a great influence on the neonate<sup>1</sup>, and infants 1 month old can detect fine differences in speech-like sounds2,3. This enables a selective response to take place when the child meets adults. From birth, babies will turn towards the source of a sound, and this orientation to a voice helps them to learn about faces. We have also observed that infants are more interested in their mother's face when she is talking.

A mother's voice is a frequent and relatively unchanging event in the young infant's auditory world, and its early

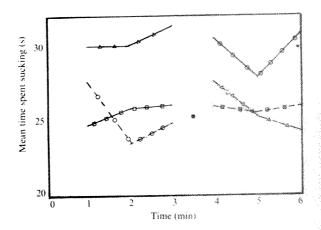


Fig. 1 Contrast effect for voice of mother (--) measured in mean time per minute spent stranger (sucking. △, Group I; ○, group II; □, group III (non-contingent). Interaction of voices and group scheduling was significant (P = < 0.05) as confirmed by analysis of variance, with repeated measure.

recognition has important consequences for the developing bond between mother and child. We report here a study which is the first designed to discover whether normal, 3-week-old infants from intact families can recognise their mother's voice. Successful recognition, we believe, is likely to aid communication.

We allowed the infants to control the auditory feedback they received (for instance, a mother's voice) by sucking on a teat to turn on the auditory stimulus. This technique, successfully employed by Siqueland et al.4 and Bruners may be used to find out what infants prefer to hear, (Operant conditioning of non-nutritive sucking has been demonstrated in neonates by Stern et al.5.) If a change in the way the infant sucks is shown to be dependent on whether it can hear the mother's voice or that of a stranger, it would indicate recognition of the mother's voice.

Babies 20-30 d old, and midway between feeds, were placed, when alert (Prechtl's Stage IV'), in an infant seat behind a screen. A blind teat placed in the mouth connected to a pressure transducer, and a light behind a transparent reading panel turned on when the infant sucked normally and alerted the mother to read. (A normal suck was 5 mm of water pressure.) Each baby heard mother's voice live and that of a female stranger equated for loudness, but saw no one. Strangers varied from baby to baby to avoid responses being due to the characteristics of one particular voice.

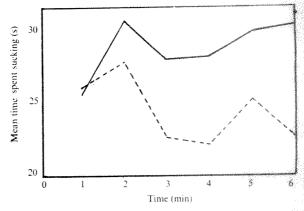


Fig. 2 Mean time spent sucking per minute during training for the contingent groups 1 and 2 combined (\_\_\_\_\_) and non-contingent group (\_\_\_\_\_). The groups and minutes non-contingent group (interaction as confirmed by analysis of variance was significant (P = < 0.05) as was the linear component of the interaction trend analysis (P = < 0.01).

Table 1 Babies discrimination between voices				
Schedule		Contingency training	Voice dis	crimination
Group 1 (n = 16 mean age 24 d, s.d. 2.6) Group 2 (n = 16, mean age 24 d, s.d. 2.6) Group 3 (n = 16, mean age 24 d, s.d. 2.2)	Base line sucking only Base line sucking only Base line sucking only	First stranger's voice contingent on sucking First stranger's voice contingent on sucking First stranger's voice controlled by non-contingent schedule	Mother's voice contingent on sucking Second stranger's voice contingent on sucking Mother's voice controlled by non-contingent schedule	Second stranger's voice contingent on sucking Mother's voice contingent on sucking Second stranger's voice controlled by non-contingent schedule 11–13 (inclusive)
Time (min)	0-1	2–7 (inclusive)	8–10 (inclusive)	11-13 (inclusive)

A non-contingent control with the same order of voice presentation as group 1 was achieved by equating total duration of feedback experienced by babies in group 1 on an individual subject basis. Schedules were generated by a computer program from the group data.

The procedure began with 1 min of sucking (baseline), and a 6 min training period followed during which the infant had a prolonged opportunity to learn the contingency between sucking and voice, and to integrate response and feedback. Data on discrimination between voices came from two further 3 min periods during which sucking produced the mother's and then the stranger's voice or vice versa (see Table 1).

The results for the voice presentation show that time spent sucking per minute and number of sucks per minute were greater (P = < 0.001 in both cases) when mother's voice was contingent on sucking. The duration of sucking for all groups is plotted in Fig. 1. No difference was recorded in the non-contingent group. Thus, the possibility of mother's voice increasing the arousal level of the infant would not account for the reported increase in sucking activity since the feedback-matching control effectively differentiates between stimulus and operant control of behaviour. Great variability in the duration of bursts of sucking in the first minute that mother's voice was available is consistent with the view that the mother's voice was initially responded to in the control group.

Non-nutritive or 'comfort' sucking typically has a rhythmic pattern in which bursts of sucking are regularly interspersed with pauses in activity. Babies could have achieved the increase in total time spent sucking when the mother's voice was available by a number of different strategies. In the event, for the mother's voice, mean burst length increased (P = < 0.025) and mean pause length shortened (P = < 0.01) as confirmed by analysis of variance. It is interesting that regulation of the sucking pattern was both systematic across contingent groups and an appropriate modification of behaviour if the mother's voice was to be found attractive.

Effects under contingent and non-contingent scheduling during the training period are plotted in Fig 2. Analysis indicates that infants were learning to produce a rewardthat of a complex auditory feedback as a result of their behaviour. Only future investigation can establish whether it is sensitivity to the contingencies inherent in the present task, or the processing of correlated information that is being demonstrated by these young infants.

Babies will expend greater effort, it seems, to hear a familiar voice. Our findings suggest that infants have already learnt some characteristics of their mother's voice, although we are not in a position to say which aspect they may be recognising. Nevertheless, successful discrimination of mother's voice is occurring before they are a month old.

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#### Pathogenicity and cerato-ulmin production in Ceratocystis ulmi

THE recognition of aggressive strains of Ceratocystis ulmi associated with the epidemic of Dutch elm disease in Britain directed attention to the importance of variation in the pathogenicity of the causal fungus1.2. British workers3.4 have provided evidence of a correlation between pathogenicity and certain cultural characters such as colony appearance and growth rate. Here I describe a correlation between pathogenicity of certain strains of C. ulmi and the production of a metabolite named cerato-ulmin which, when introduced to elm seedlings, is capable of producing many of the symptoms of the disease.

Cerato-ulmin was previously described as microstructures<sup>6</sup> which are produced abundantly in liquid shake cultures of C. ulmi. It has unusual characteristics. As already reported5.6 these

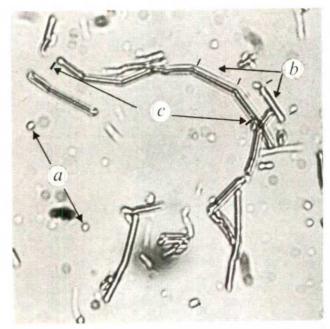
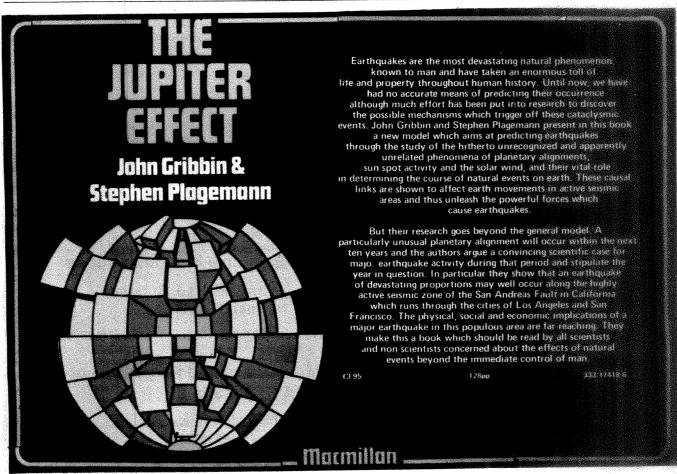


Fig. 1 Three unstable forms of cerato-ulmin in water as observed under the microscope. a, 'Unit' (a primary form); b, 'rod'; c, 'fibril' ( $\times$  256). ('Rod' and 'fibril' are assemblages of 'units.')



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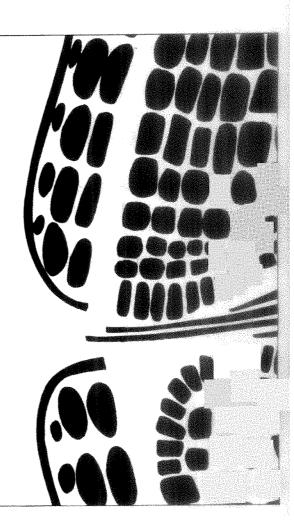
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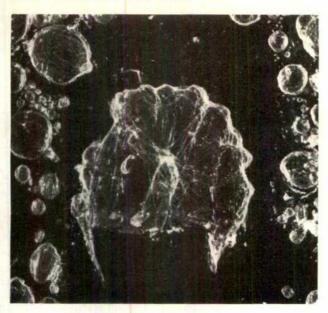


Fig. 2 By bubbling or vacuum effervescing (S.T., unpublished) rods and fibrils are forced on to the surface of the liquid as bubbles. When these accumulated bubbles burst, circular pieces of membrane, as shown here, remain floating on the liquid surface (× 30).

microstructures are of unstable form, and readily change from units' to 'rods' and 'fibrils' (Fig. 1). As 'units' the solution remains clear, but once 'rods' and 'fibrils' are formed the solution becomes milky in appearance. An additional assemblage of cerato-ulmin is 'membrane' (Fig. 2). After centrifugation, however, the fluid becomes clear. Such changes in morphology of cerato-ulmin in water indicate that it may be a liquid crystal, specifically a lyotropic liquid crystal. Cerato-ulmin seems to possess both carbohydrate and peptide moieties in its molecule.

Cerato-ulmin is certainly released into the culture medium but the actual production site has not yet been confirmed. When crushed spores which were repeatedly washed with water to remove cerato-ulmin from the spore surface were extracted, however, no cerato-ulmin has detected in the extract. It is therefore assumed that cerato-ulmin is produced extracellularly.

When five *Ceratocystis* species other than *C. ulmi* were cultured in the same conditions as those in which *C. ulmi* was grown to produce cerato-ulmin, no cerato-ulmin was detected. These species were *C. dryocoetidis* Kendr. and Moln., *C. fagacearum* (Bretz) Hunt, *C. major* (von Beyma) *C. Moreau*, *C. minor* (Hedge.) Hunt and *C. piceae* (Münch) Bakshi. Thus, cerato-ulmin seems to be a specific metabolite for *C. ulmi*.

Cerato-ulmin began to appear in liquid shake culture 3 d after inoculation, while active spore production was continuing and eventually reached 70% of the maximum level. Subsequently, the production of cerato-ulmin was rapidly accelerated but this accelerated production ceased 13 d after inoculation. Spore production was fluctuating at this time following peak production 5 d after inoculation. Beyond 13 d cerato-ulmin remained constant in spite of a levelling off of the spore number. This indicates that cerato-ulmin is a metabolite during active fungal development. In addition, even when a sharp reduction in spore numbers in the culture—probably indicative of autolysis—took place 22 d after inoculation, no change was detected in the level of cerato-ulmin. So cerato-ulmin is not considered an autolytic product of the fungus.

Cerato-ulmin causes white elm (*Ulmus americana* L.) seedlings to display symptoms similar to those produced by Dutch elm disease. A cerato-ulmin aqueous solution (120 mg ml<sup>-1</sup>) was continuously pressure injected (351.5 g cm<sup>-2</sup>) into the cuttings of 3-yr-old white elm seedlings in greenhouse conditions. An initiation of a substantial reduction in uptake appeared after 3 h; obvious drooping of leaves after 19 h; irreversible wilting and chlorosis on living leaves, after 43 h; and necrosis

Table 1 Correlation between production of cerato-ulmin and pathogenicity of C. ulmi

Strain	Spore produc- tion ml <sup>-1</sup> (×10 <sup>6</sup> )	Index of cerato-ulmin	Indication of pathogenicity†
CECC 121/	1.267	production*	
CESS 13K	1,267	265	+8
CESS 14K	1,266	118	+8
CESS 16K	1,426	278	+8
CESS CH5	1,536	• 118	+8
CESS PM1	1,323	140	+8
CESS T3	1,281	140	+ §
CESS W1	1,283	300	+§
P1	949	1	士§
Q412	1,369	8	± §
W2 (Gloucestershire)	‡ 1,066	304	+1
W4 (Essex)‡	1,047	337	+1
W7 (Cambridge)‡	1,313	6	+1
W9 (Oxfordshire)‡	1,367	6	<b>E</b>

Cultural conditions were the same as reported previously except that the level of the carbon source (sucrose) was shifted to 2%. Age of cultures was 1 week. Turbidity was measured at the wavelength of 400 nm using B & L Spectronic 20.

\*Calculated as (turbidity) × (dilution factor of the sample for measurement) × 100.

†Expressed as + (highly pathogenic) or  $\pm$  (weakly pathogenic).

‡British strains.

§After Kondo (unpublished).

After Gibbs and Brasier<sup>3</sup>.

on living leaves after 91 h. When the xylem was checked for brown discoloration at the end of the experiment (168 h after injections were begun), it was detected in all treated cuttings. No abnormal symptom was found in the control cuttings injected with distilled water however.

As cerato-ulmin seemed to be significant in disease development, the elucidation of the relationship between ceratoulmin production and the pathogenicity of C. ulmi strains was of great interest. There seems to be no correlation between spore production and either cerato-ulmin production or pathogenicity (Table 1). Production of cerato-ulmin was found to be either high or low according to the method of measuring used. On this basis, C. ulmi strains were divided into two obviously different groups: high production and low production. A striking fact is that all strains with high cerato-ulmin production were in the highly pathogenic group, while all strains with low cerato-ulmin production were in the weakly pathogenic group. This relationship seemed clear-cut with no overlap between the two groups on the basis of cerato-ulmin production and pathogenicity. It should be noted that, of the four British strains, two are highly pathogenic (aggressive) and two are weakly pathogenic (non-aggressive)3.4; these clearly separated in exactly the manner described above. Although there is variation in the production of cerato-ulmin in the high-production group of strains, it is not clear if intermediate production represents moderate pathogenicity of the strain because comparative data are insufficient for testing pathogenicity of those strains.

The ability of cerato-ulmin to produce symptoms of toxicity in white elm seedlings similar to those produced by Dutch elm disease suggests that cerato-ulmin could be responsible for disease symptom development in infected elm trees, if one assumes that the metabolite is produced in vivo. If this is the case, certain factors involved in cerato-ulmin production are inclined to induce symptom development. It is produced extracellularly and this ensures that there is good contact between cerato-ulmin and host tissues; because it is specific for C. ulmi it can direct specific symptom development, and its production during active fungal development is linked to its rapid production by the actively developing fungus within the host tissues. (Active development of the fungus is generally supposed to be dominant at the early stage of disease development.)

Together with the ability of cerato-ulmin to produce symptoms similar to those of Dutch elm disease, the correlation between cerato-ulmin production and pathogenicity indicates that differential production of cerato-ulmin is possibly responsible

for variations in pathogenicity among C. ulmi strains. On this basis, the production of cerato-ulmin might be a very useful criterion for fungal pathogenicity. Work is in progress to ascertain if it is applicable in a wider range of strains of C. ulmi than are presented here.

I thank Dr J. N. Gibbs and Dr G. F. Ouellette for cultures of their isolates, Dr E. S. Kondo for permission to use his data concerning the pathogenicity of C. ulmi strains in our collection, and Mr W. C. Richards for technical help.

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#### Stomatal movements and ion fluxes within epidermis of Commelina communis L.

STOMATAL movements are brought about by differential changes in turgor pressure between guard cells and other epidermal cells1,2. The mechanism by which the turgor changes occur has been investigated for well over a century and a number of hypotheses have been proposed but none has been found to be completely satisfactory. Research in recent years has provided increasing evidence that active ion transport plays a major role in the stomatal mechanism3-6. It has been shown that, as stomata open, certain ions move into the guard cells and that this ion influx may be a dominant factor in raising the guard cell osmotic concentration. As a result, water enters the guard cells and brings about the opening movement. During closure of stomata the process seems to reverse whereby ions are lost by the guard cells. Cl- and K+ seem to be the major ions involved in the stomatal mechanism4.6-10

Humble and Raschke10 observed that in guard cells of Vicia faba the CI equivalents were considerably less than were needed to balance the K equivalents; a similar situation existed in the guard cells of Zea mays although the K-Cl imbalance was not nearly so great11.

Although Commelina communis has been used intensively for studies into stomatal mechanisms, assessment of the ion fluxes and charge balances involved have been neglected. Here we examine the fluxes of K, Cl, Ca and P between guard cells, subsidiary cells and epidermal cells of Commelina communis L. during stomatal movements. The results are discussed in relation to the K-Cl balance within the cell types in a closed and open stomatal apparatus.

Leaves from C. communis grown in the greenhouse in a peat-vermiculite mix supplemented with a complete fertiliser were used in the experiments. To obtain open stomata, the youngest, fully expanded leaves were floated on deionised water, abaxial surface downwards and illuminated from above for at least 3 h. Closed stomata were obtained by placing potted

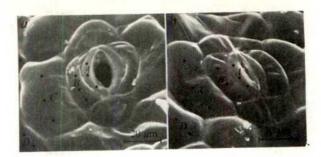


Fig. 1 Scanning electron micrographs of, a, an open and, b, a closed stoma of C. communis showing the approximate positions of point analyses in, A, a guard cell; B, an inner lateral subsidiary cell; C, an outer lateral subsidiary cell; and, D, epidermal cell.

plants in a darkened cupboard for 3 h. Before the epidermis was detached the leaves were examined under a microscope at low power (x 100) to ensure that the stomata were open or closed. The abaxial epidermal strips, cuticle side uppermost, were placed on to carbon disks and rapidly frozen in liquid nitrogen. The epidermis, still attached to the carbon disks, was then freeze dried for not less than 60 min at  $-40^{\circ}$  C under a vacuum of 0.05 mm mercury. After coating with carbon (approximately 100 µm thick) a dispersive-type analysis for the elements was made in the various cells of the epidermis (Fig. 1) using a scanning electron microscope with a solid-state X-ray detector attachment (Acton Ms-64 electron probe). The microscope was operated at 18 kV accelerating voltage with a sample current of approximately 100 nA. The take-off angle was 18°.

After obtaining secondary electron images of the stomata (Fig. 1), spot analyses (100 s counts with a spot size of 1 μm) were made at three positions (Fig. 1) in each of the following cells; guard cell, inner lateral subsidiary cell, outer lateral subsidiary cell and epidermal cell. This procedure was repeated three times for an open stoma (13 µm aperture) (Fig. 1a) and a closed or nearly closed stoma (>1 µm aperture) (Fig. 1b). Potassium X-ray intensity slowly decreased with time due to volatilisation and so care was taken to initiate counting at the same relative time on each point. Thus, the error inherent in an analysis was comparable in each measurement.

Solid state X-ray spectrometers (used in this study) lack the energy resolution of the crystal spectrometers of the electron-microprobe analysers and although they measure through the whole spectrum simultaneously there is some interference between elements being measured. In efforts to minimise this the specimens were coated with carbon rather than metals which are normally used to obtain secondary electron images.

Both Ca and P are considered to be relatively immobile12, a contention supported in these studies by the relatively constant

Table 1 Levels of K, Cl and Ca based against cell P levels and the K-Cl ratios in different cells of the epidermis of C. communis when the stomata were open (13 µm) and virtually closed (>1 µm)

Ratio	Guard cell	subsidiary cell	Outer lateral subsidiary cell	Epidermal cell
		Open stomata	L	2 20 1 0 40
K-CI	$1.29 \pm 0.16$	$1.65 \pm 0.44$	$1.99 \pm 0.72$	$2.20 \pm 0.60$
Ca-P	$1.83 \pm 0.36$	$1.92 \pm 0.38$		
K-P	$5.37 \pm 0.83$	$2.37 \pm 0.38$	$2.43 \pm 0.71$	$3.04 \pm 0.79$
CI-P	$4.22 \pm 0.85$	$1.47 \pm 0.21$	$1.24 \pm 0.10$	$1.42 \pm 0.28$
CII		Closed stomat	a	
K-Cl	$1.88 \pm 0.15$	$1.97 \pm 0.33$	$2.47 \pm 0.50$	$2.99 \pm 0.44$
Ca-P		$1.93 \pm 0.59$	-	
K-P	$3.21 \pm 0.41$		$3.75 \pm 0.67$	$4.03 \pm 0.56$
CI-P	$1.70 \pm 0.18$	$1.43 \pm 0.15$		$1.38 \pm 0.27$

Each figure represents the mean of nine determinations with its accompanying standard deviation.

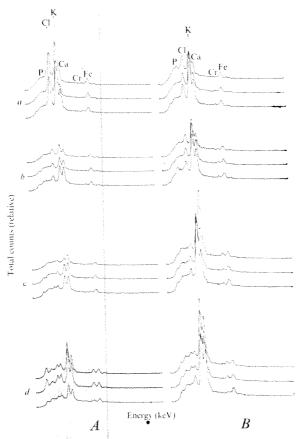


Fig. 2 Representative X-ray spectra of a microprobe analysis (three points per cell) of, a, a guard cell; b, an inner lateral subsidiary cell; c, an outer lateral subsidiary cell; and, c, an epidermal cell of C. communis with an open stoma, A, and a closed stoma, B. The Cr and Fe peaks are from the pole piece of the microscope.

Ca-P values observed in guard cells and inner lateral subsidiary cells when stomata were open and closed (Table 1). Ca-P ratios for the outer lateral subsidiary cells and epidermal cells could not be obtained because these cells contain large crystals of calcium oxalate and frequently the electron beam would hit a crystal and send the counts off scale (Fig. 2c, column B). Since the sample current varied slightly for specimens with open and closed stomata, peak heights for each of the elements could not be directly compared. Therefore, the K and Cl peaks were based against the P peaks which were considered constant from cell to cell. By expressing the K and Cl levels against P (which acts as a 'standard') errors which would be caused by the increase in guard cell volume when stomata are open are also largely eradicated.

Figure 2 shows representative X-ray spectra of the different cell types associated with an open stoma, A, and a closed stoma, B. A qualitative picture of the change in the levels of the different elements in each of the cell types associated with the open and closed stomata can be deduced from the peak heights.

Table 1 bases the peak heights of the elements against the P 'standard' enabling a more quantitative picture of the levels of the various elements within the cell types to be obtained. Highest levels of K were observed in the guard cell of the open stoma with least in the neighbouring inner and outer lateral subsidiary cells. In the case of the virtually closed stoma, K levels in the guard cell decreased relative to the open stoma while levels in the inner lateral subsidiary cell, and particularly the outer lateral subsidiary cell and epidermal cell, increased. Except for the guard cell of the open stoma which possessed the highest Cl levels, Cl levels were similar in all the cell types regardless of whether the stomata were open or closed. Thus, when a stoma of C. communis of aperture 13 µm closes to about 1 µm in response to darkness, K moves out of the guard

cell setting up a flux across to the epidermal cell. The revers flux would occur upon opening. It would be presumed that Cl follows a route similar to that of K although, in thes studies. Cl levels were not observed to increase in subsidiar and epidermal cells upon stomatal closure. Possibly Cl move rapidly to other epidermal and/or mesophyll cells and does no accumulate in the subsidiary cells as K does. Only when for reach epidermal cells are they able to enter mesophyll cell directly since the sub-stomatal cavity underfies guard an subsidiary cells.

Such a pathway for K (and possibly Cl) movement pose questions regarding the position(s) of the ion pump(s). pump system presumably could be located in the guard ce plasmalemma which would maintain K gradients between it an the adjoining cells. Alternatively, a pump could be locate in each cell type. A further consideration is whether the ior move between the cells through plasmodesmata and/or throug the cell-free space.

Thus, the ion fluxes in C. communis do not seem to b similar to those in maize in which there is a movement of I and Cl between a pair of subsidiary cells and guard cells durin stomatal movements11. The grasses in general, in fact, seem t possess a K shuttle between guard cells and subsidiary cell during stomatal movements13.

K-Cl ratios in the different cells types increased in the orde of guard cell < inner lateral subsidiary cell < outer latera subsidiary cell < epidermal cell. This order was maintaine regardless of whether the stoma was open or closed (Table 1 Thus, there was a greater inbalance of the two ions in epiderina cells. This inbalance of K-Cl also tended to be greatest in a the cells associated with the closed stoma. If organic aci anions bring about electrochemical neutrality balancing th excess K, as Humble and Raschke10 have suggested in epiderma cells, then it would be expected that the production of suc anions must be even greater in epidermal cells. Evidence so to does not support this contention, however, since enzyme involved in the production of malate and certain other organi acids seem to predominate in the guard cells rather than the epidermal cells14. Presumably, however, organic acid flux from guard cells to the epidermal cells could overcome th

We thank Drs J. Brown and J. Johnson of Georgia Technica University for their help in the operation of the microprobe.

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#### Carbohydrate-binding protein from Polysphondylium pallidum implicated in intercellular adhesion

INTERCELLULAR adhesion is generally believed to be mediated by specific macromolecular components on the surface of the interacting cells. Soluble factors which may play a part in this process have been identified in a number of systems1-8. We have already described a developmentally-regulated carbohydrate binding protein 10,11 from the cellular slime mould Dictyostelium discoideum that appears in differentiating amoebae in close correlation with the development of cohesiveness10. This protein is assayed by its erythrocyte agglutination activity that can be inhibited by specific sugars. We now report the isolation of a similar protein from another cellular slime mould, Polysphondylium pallidum that seems to mediate specific cellular adhesion as judged from the following findings: (1) it is present on the cell surface; (2) it is present when the cells are differentiated to a cohesive state but absent when the cells are not cohesive; (3) addition of the purified protein promotes cell cohesion; (4) sugars which react with the active site of the molecule block cell cohesion produced either by the added purified substance or by the endogenous substance present on cohesive cells; (5) the precise reactivities of the protein from P. pallidum differ from the protein from D. discoideum, which correlates with the segregation of these cells in mixed culture.

P. pallidum, like the other cellular slime moulds, exhibits two distinct phases in its life cycle<sup>12-14</sup>: a non-social vegetative phase and a cohesive phase, initiated by starvation. To generate vegetative and cohesive cells, 105 spores of P. pallidum WS-320 in association with pregrown Aerobacter aerogenes were inoculated on to standard medium (SM) agar plates<sup>15</sup> (100 mm diameter) and incubated in a moist atmosphere at 22° C in the dark. Cells in the growth phase (or not far from it) were collected from plates after 63 h of incubation when their density was fairly low (7 × 10<sup>7</sup> per plate), washed in cold H<sub>2</sub>O and separated from bacteria by centrifugation. The amoebae were differentiated16 into the aggregation-competent state by suspension (10<sup>7</sup> ml<sup>-1</sup>) and reciprocal shaking in 0.0167 M Na<sub>2</sub>HPO<sub>4</sub>-KH<sub>2</sub>PO<sub>4</sub>, pH6.0. At intervals cells were removed for assay of cohesiveness and to be extracted for erythrocyte agglutination activity (Fig. 1). The cohesiveness assay, modified from one described previously10, measures the size of clumps formed from single cells passively brought into contact by roller-tube agitation. It has been demonstrated 20,21 for D. discoideum that the size of free suspension agglutinates formed from single cells in the presence of EDTA correlates with the ability of the cells to form morphogenic contacts.

**Table** Sugar effects on agglutination of erythrocytes by discoidin or *P. pallidum* agglutinin

		for 50% inhibition of
Sugar	agglutina	ation (mM)
	P. pallidum agglutinin	D. discoideum agglutinin
Lactose	1.6	12.5
Methyl-D-galactose	6.2	6.2
D-Galactose	6.2	25
N-acetyl-p-galactosam	ine 25	1.6
D-Fucose	25	3.1
-Fucose	50	12.5
3-O-methyl-D-glucose	100	1.6
a Methyl-D-glucose	≥ 100	12.5
3 Methyl-D-glucose	≥ 100	100
<b>&gt;-Glucose</b>	≥ 100	≥ 100
o-Glucosamine	≥ 100	≥ 100
N-acetyl-D-glucosamir		≥ 100
>-Mannose	≥ 100	≥ 100
Methyl-D-mannose	≥ 100	≥ 100
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Purified P. pallidum agglutinin and discoidin<sup>11</sup> were assayed for agglutination activity as described in Fig. 1 in the presence of a eries of concentrations of sugars to determine the sugar concentration required to inhibit agglutination activity by 50%.

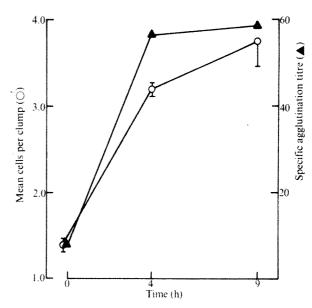


Fig. 1 Agglutinin activity (▲) and cohesiveness (○) during development of *P. pallidum* in suspension culture. Cells maintained on SM plates<sup>15</sup> with bacteria for 63 h were washed free of bacteria and inoculated in suspension medium without bacteria<sup>16</sup>. Aliquots of differentiating cells were removed for assay at the times indicated. For the agglutination activity assay extracts were prepared by sonicating  $3 \times 10^8$  cells (Bronwill Sonifer with needle probe; four bursts of 25 s each at intensity 50) in 3 ml of 75 mM NaCl, 75 mM KCl, 1 mM EDTA, 15 mM Tris-HCl (pH 7.3)<sup>17</sup> containing 0.3 M D-galactose. Extracts were centrifuged at 150,000g for 45 min (4° C). Supernatants, after extensive dialysis against 75 mM NaCl, 75 mM Na<sub>2</sub>HPO<sub>4</sub>-KH<sub>2</sub>PO<sub>4</sub> (pH 7.2) (PBS) were assayed with formalinised human red blood cells<sup>18</sup> (FHRBC). Assays were performed in Microtiter V plates (Cooke Engineering) using serial twofold dilutions of the extract. Each well contained 25 µl of saline (0.15 M NaCl) plus 25  $\mu$ l of extract diluted in PBS to which 25  $\mu$ l of a 2.5% (v/v) suspension of FHRBC in PBS was added. The patterns were read after 1.5 h. Titre was expressed as the reciprocal of the highest dilution giving positive agglutination. Specific agglutination activity was expressed as titre divided by protein concentration. Protein concentration was determined by the method of Lowry et al.19 with bovine serum albumin as a standard. For the cohesiveness assay the procedure was as follows: (1) Cells collected from the suspension culture were washed in water (4° C) and suspended in EDTA-phosphate buffer (16.7 mM Na<sub>2</sub>HPO<sub>4</sub>-KH<sub>2</sub>PO<sub>4</sub>, 10 mM EDTA (pH 6.2)) as modified from Gerisch<sup>20</sup>. (2) The suspension was dispersed into single cells by repeated pipetting through a fine-tipped pipette and was adjusted to a concentration of  $5 \times 10^5$  cells ml<sup>-1</sup>. 1 ml of this suspension was diluted 50-fold in EDTA-phosphate buffer and counted with Coulter counter (Model ZB-I) using a 200 µm aperture with 1/aperture current at 1/16, 1/amplitude at 1/8 and the lower and upper thresholds set at 20 and  $\infty$ . (3) 5 ml of the cell suspension was added to a  $17 \times 150$  mm glass test tube. The tube was rolled about its long axis at 20 r.p.m. for 30 min (23° C). (4) The contents of the tube were diluted 20-fold in EDTA-phosphate buffer and counted in the Coulter counter at the above settings. With coincidence corrections, the ratio of the total number of particles before and after rolling was computed. This ratio is the mean number of cells per clump and is taken as an index of cell cohesiveness. The standard error of the mean for three separate determinations is shown.

Cohesiveness of cells and specific agglutination activity in cell extracts increased in parallel after separation from bacteria (Fig. 1). In subsequent experiments, we found that synchronous growth-phase cells grown in suspension with bacteria the under conditions in which all cells had equal and ample bacterial food, contained no detectable agglutination activity. Thus agglutination activity was absent in vegetative cells, but appeared in food-deprived, differentiating cells.

To purify agglutinin we cultured cells on SM plates for 90 h in the dark. Under these conditions the cells become cohesive but do not develop beyond broad, loose aggregates. We sonicated the cells in 0.15 M NaCl, 0.3 M galactose, adsorbed the dialysed supernatant with formalinised erythrocytes and (Continued on page 149)



# Book Revieu Supplement

THE prefix 'para—', says the OED, means 'beside, beyond, wrong, irregular''. Excellent—just right for the terms 'parascientist' and 'parathinker'.

Tact prevents my naming presentday parascientists and parathinkers: but anyone can see that some of them at least must be identified by the field of intellectual endeavour in which they oprate, that is to say in interpreting what I shall call the 'it-makes-you-think-don't-it?' type of seemingly natural occurrence such as being given one's grandmother's maiden name by a clairvoyant, seeing Uri Geller bend teaspoons, or guessing (beyond statistical prediction) what the next card but one is going to be that somebody turns up in the next room. If the last of the three actually does happen to hold water from a statistical point of view, it remains egregiously boring and useless: it doesn't lead to anything. And the first two are drawn from a field of human activity that is, alas, far too wide-open to illusion, charlatanry and

But the serious point I want to make is something other, something which underlines my scorn for parascientists and parathinkers. It's this. So much of the interpreting, let alone the theorising, of parascientists and parathinkers is based on seemingly natural occurrences which either cannot be fully corroborated according to the rules of evidence, or cannot be properly repeated according to the rules of experiment-or, of course, both. Yet this is not to say that to me the most striking thing about parascientists and parathinkers is their being beside, beyond, and so on; lots of scientists and thinkers have been all of those. (Think of Newton on numerology.) What to me seems the most striking thing about them is what makes them lurable into the beyond, beside, irregular and wrong: namely an astonishing credulity. The critical link between 'it-makes-youthink' and 'there-must-be-something-in-

### All parasense

William Cooper

Aldous Huxley: A Biography Vol. 2: 1939–1963. By Sybille Bedford. Pp. xii+378+15 photographs. (Chatto and Windus, in association with Collins: London, 1974.) £4.50.



Aldous Huxley. A portrait by Alfred Wolmark, drawn in 1928. (National Portrait Gallery)

it' seems to me indefensibly weak, if not totally absent. I don't understand it: they include some very clever men. Here speaks one of them:

"Elinor Bond doing telepathic guessing ... Frances Farrelly, with her diagnostic machine ... Harry, the Dutch sculptor, who goes into trances in the Faraday cages and produces automatic scripts in Egyptian hieroglyphics; Narodny, the cockroach man, preparing experiments to test the effects of human telephathy on insects ... It was all very lively and amusing and, I really think, promising ..."

All very lively and amusing is for him to say, naturally. Promising—we can have an opinion on that. The only think it surely promises is more of the same nonsense. The speaker is Aldou Huxley.

Now we all have our off-moment and the quotation, which comes from the second volume of Sybille Bedford biography of Aldous, may not be er tirely fair. But it is not entirely unfair The part played in the second half c Aldous's life by-shall we call it?parasense, in whatever degree of light heartedness or seriousness, is too grea to allow that he be absolved from th charge of astonishing credulity. It i clear that he, being one of the mos intelligent of men, was singularly fre from pomposity, ill-will and aggressive ness: all his friends loved him as self lessly thoughtful and harmoniou almost to the point of saintliness. Th condition of men in the twentiet century, Isaiah Berlin observes, wa the single theme that dominated hi late years. And beyond warnings to mankind-first of nuclear holocaus and then of ecological catastrophe-Aldous's message for each of us, per sonally, delivered with a most taking humility and amused down-to-earth ness was: Try and be a little kinder to each other. Hard to resist, I must say. True, there was something remote about him: as everybody has pointed out, he scarcely noticed that his first wife, Maria, was dying of cancer. (Or the other hand he too died of cancer with great dignity.) Yet . . . why did he allow himself to be so credulous about paranormal occurrences, about 'mind-changing' drugs and the like? The only person to give any sort of answer is Charles Snow, who suggests "just one touch of immodesty. . he kept his mind open beyond the limits of openness, could he perhaps happen on something earth-shaking, altogether outside the common run of life? And enjoy announcing it to an astonished world? Like a more dramatic T. H. Huxley about something more exciting than Darwin's

Theory?" It's hard to come up with a more likely answer. Poor Aldous: it never came off; it never could.

So Mrs Bedford arouses our compassion. Over my regard for her as a biographer I shot my bolt when reviewing Volume 1 in this journal. I see myself quoted on the dustjacket of Volume 2: "Mrs Bedford has great literary talent and through everythink she writes shines a sympathetic intelligence-not to say an admirably unforced professionalism as well". I stick to that. Over a final verdict on the biography I naturally insisted on waiting until the end. The doubts I had at that time have not really been resolved now that I have read the second volume. I noticed that the book has the flavour of close, cosy, penetrating gossip: all well and good-but I feel that in the end it becomes too much of a good thing. There are too many gossippy letters from Maria, too many long quotations from other people's letters. And for my taste there is too much detail-Joe Blodgett'ish, American academic, research detailabout Aldous's incessant, restless lecturing tours. (Did he really have to travel so much, and to live in southern California, in order to make a living and maintain his health? Such a life was particularly bad for him; he ought to have stayed put, among more stableminded men of his own intellectual stature, in a less fatuous culture.)

Finally, I get a recurrent feeling that Mrs Bedford, who knew Aldous well and was a close friend, is somehow in this volume shielding him, protecting him from criticism. It's wholly understandable, and it's permissible for part of the time; but there's another part of the time when a biographer must come clean, and cold—or so it seems to me. All the same, Mrs Bedford has written a remarkable and valuable book

# Science from the beginning

Early Physics and Astronomy: A Historical Introduction. (History of Science Library.) By O. Pedersen and M. Pihl. Pp.413. (Macdonald: London; American Elsevier: New York, August 1974.) £10.95.

THE content of this revised and updated English version of an originally Danish text is summarised well in the description on the dust jacket of the book. "The first section of the book is devoted mainly to Greek physical science... Concepts of nature and of scientific laws in general are discussed in addition to theories within particular sciences. The reader is also introduced to the mathe-

matical and other techniques available for the solution of problems. The second section of the book discusses the transmission, reception and elaboration of these theories in the Latin West, and the science of the early Renaissance is seen as the end-point of the classical tradition. The book concludes with biographical sketches of the philosophers and scientists featured in the text."

Not everyone would agree with the authors' prefatory remark that their omission of a detailed account of early Egyptian and Babylonian science was "inevitable", though it was doubtless expedient for them to limit their already extensive theme. Probably for the same reason they omitted any mention of the rich scientific developments in mediaeval India or China, the early history of pure mathematics, time-reckoning, cartography, and so on. It is, on the other hand, truly inevitable, and a little unfortunate, that Alexander Thom's important researches on megalithic lunar observatories and solstitial sites have been published too recently for inclusion in the text or in the bibliography (updated to 1970).

In the treatment of Greek science emphasis is laid upon the importance of the presocratic belief that causal relationships existed between natural phenomena. Aristotle's adherence to this causal principle (or natural law), coupled with the trend away from mythology towards rational explanation and the Platonic concept of the mathematisation of nature, constituted essential ingredients of subsequent scientific thought. Menaechmos's discovery of the conic sections (in about 350 BC) may have arisen from the study of the daily shadows cast by a gnomon upon the ground. That was a hidden empiricism which is also detectable in the Pythagorean relationships between number and harmony, the Method of Archimedes, and the pneumatical experiments of Strato Philo and Hero at Alexandria during the Hellenistic period of Greek cul-

It was, however, mediaeval investigations in optics and statics, founded upon principles drawn from everyday experience, which provided the firmer empirical basis of physical science. The plane astrolabe and mechanical clock were but two of many mediaeval inventions destined to revolutionise astronomical practice; and the rejection of the Aristotelian belief in a causal principle for the explanation of motion was the determinative factor in the development of dynamics, which was required to make the physical implications of Copernican astronomy acceptable to Galileo and many of his contemporaries.

The field which is explored is much more complex and extensive than this outline may suggest, and is already familiar to students of early science. The well balanced presentation in one volume is a welcome substitute for the wide range of scattered publications from which the subject matter would otherwise have to be drawn. The extensive bibliographical and biographical references provide a very useful guide to carefully selected primary and secondary sources. The use of vector notation to simplify explanations of the various geometrical devices used by Greek and mediaeval scholars, though a useful heuristic tool, is anachronistic and presupposes that the reader has received instruction in this technique.

I detected only six minor printing errors in the text, but slightly less care seems to have been taken by the authors and publishers in connection with the description of a great many of the accompanying figures.

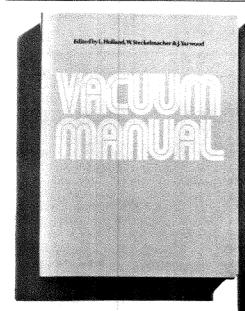
The authors' hope that this book will become widely used as a standard text may be dashed because of its price. The onus is likely to fall upon teachers to obtain library copies and to arrange that these are easily accessible to the members of their classes. My own experience leads me to believe that the clear and scholarly style of this excellent translation, along with the authors' practice of breaking up the text into numerous sub-sections, will appeal greatly to science undergraduates reading the book as part of an introductory survey course in the history of the exact sciences.

Eric G. Forbes

# No matter—never mind

The Brain Revolution. By Marilyn Ferguson. Pp. 380. (David-Poynter: London, 1973.) £4.00.

This book reviews the flotsam and jetsam discovered by those working on the remote shores of the brain sciences. Among other things, it tells us that "human volunteers in biofeedback laboratories are learning consciously to control their brain waves, perspiration, blood pressure, digestive juices and heart rate" (p.17): anyone intending to read it would be well advised to acquire such control first. The authoress tells us that "breakthrough scientists ask silly 'questions" (p.286) and the book parades before us their silly answers. The human psyche is said to be influenced by star patterns, magnetic fields and negative ionisation; precognition and telekinesis are accepted scientific phenomena; the "incredible potency of LSD" (p.123) cures



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psychoses, autism and alcoholism (though according to one study the last cure only occurs "in 50% of cases" since only one out of the two alcoholics treated actually improved, p.124); we can perceive colour with our finger tips when we are not using them for curing cancer by electrical emanations; and we can of course learn effortlessly either by eating "respected scholars" or by having linguaphone records played to us whilst we are asleep.

The language in which the book is written suggests that the authoress may herself have been in ASC (altered state of consciousness) throughout its preparation. Scientists are alternatively "stunned" and "baffled"; discoveries are "astounding", "amazing", "incredible" or "one of the biggest breakthroughs of our times". Her style speaks for itself: "Into the whirlpool of eddying, oscillating, hormones of a human female toss a contraceptive pill. The vigilant brain, taking astonished note of the suddenly escalated progesterone and oestrogen, behaves as if pregnancy has occurred" (p. 219).

Even when she deals with well established phenomena, such as size constancy, the limits of the visible spectrum, or the after-effects of seen movement, Marilyn Ferguson can do no more than invite us to gawp at them: no attempt is made to provide the reader with an understanding or an explanation, and mysteries are conjured up where none exist. In spite of reservations about the way she presents her material, one cannot but admire her industry: there is a 23 page bibliography in which the citations range from Science, through Esquire to the Psychedelic Review.

Although this book cannot be taken seriously, it may be worth asking whether it matters that so much effort is devoted to making specious discoveries and publicising nonsensical theories: after all, the brain may work in ways that are at variance with existing scientific paradigms, and unless someone produces and tests unlikely hypotheses, we would never know. It may even be that some of the unusual findings reported in this book will eventually be validated. Moreover, in the long run science will catch up and false hypotheses and observations will be discarded. In the meantime, it is only the gullible, those in search of a mystery or a sensation, who will give credence to this kind of work: the rest of us either suspend judgement or hear on the grapevine about all the negative results that do not get into the

Sensation mongering research often does, however, have bad effects. First, much of it, particularly in Russia and the United States is funded from public

money which might be better spent elsewhere. Second, scientists may feel a duty to investigate claims made by others, and if the original research is bad this may waste a great deal of time and effort: proving that an effect does not exist is notoriously difficult. It is said that a Nobel prize winner spent three years attempting to replicate some of the experiments on cannibalism in planaria without success. Third, current trends to mysticism are given a scientific prop. Not all mysticism is bad but mysticism based on bad science is likely to be bad mysticism. Fourth, and most important, when the 'discoveries' are in the brain sciences, they often fall into the hands of medical practitioners, the most gullible of professional people; and when applied to human patients such discoveries can cause a great deal of suffering. Leeches and calomel have now



Ernst Mach (sporting a splendid moustache) lying on his back and sketching the outside world as it presents itself to his left eye. From Logic, Labels, and Flesh. By Stefan Themerrson. Pp. 202. (Gaberbocchus: London, July 1974.) £3.60 boards; £2.40 paper.

been abandoned, prefrontal leucotomies and insulin-induced coma are on the decline, but psychoanalytic therapy still thrives in the face of the evidence, and there is an increasing fashion for amygdaloid lesions and for maintaining children with that mysterious syndrome "minimal brain damage" on large doses of amphetamine.

Perhaps the research councils should establish teams of sceptical but tolerant scientists prepared to move to trouble spots and investigate, in collaboration with the original discoverers, any seemingly outrageous claims that might be of importance either for science or human life. No doubt, on most occasions, they would merely put out the flames expeditiously, but occasionally a really important discovery might be rescued from scepticism, and

at least we might have firm answers to such questions as whether acupuncture works by some process other than blind faith, or whether learning can be transferred from one animal to another by injecting a homogenised brain extract. It does seem extraordinary that no definitive answer to this last question can be given 15 years after it was first raised.

N. S. Sutherland

#### Neuroteleology

Logic of the Living Brain. By Gerd Sommerhoff. Pp. ix+413. (Wiley: London and New York, 1974.) £5.75.

In 1950 Sommerhoff published a book entitled Analytical Biology: it deserves to be more widely read than it has been since it is one of the best philosophical analyses of teleological terms. He argued that a system may be said to be goal directed when its outputs correlate with variations in the states of the environment in such a way that for a wide range of environmental states the same end condition is achieved. He named this concept 'directive correlation' and tried to formalise the idea. His new book attempts a grandiose synthesis of brain and behaviour, and had it too been published 24 years ago, it would doubtless have met with the measure of critical acclaim that in that distant age greeted other pot-pourris of the kind, such as Hebbs' Organisation of Behaviour. Like such books, it would also have been forgotten by now.

In the first section of his new book, Sommerhoff argues that biologists and psychologists have been unable to grapple with the real complexity of the brain because they have not had a set of objective scientific concepts appropriate for the description of a system that is goal directed and that forms and uses models of the outside world. He alleges that brain scientists, by avoiding all teleological language, either oversimplify the problem of how the brain controls behaviour or else they use teleological language in a confused and non-objective way and thereproduce muddled theories. fore Unfortunately, no instances are given of the muddles that arise and one can only speculate about who all these confused theorists are.

Sommerhoff's solution to this problem is two-fold: first, to make use of his earlier idea of directive correlation; and second, to construct nerve nets with classificatory functions that can change through learning. In the second half of the book, he looks at various behavioural phenomena and tries to show how they can be explained in terms of these two ideas.

The book has several serious faults:

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First, it is often wildly inaccurate. For example, it is stated that "The assumption that reinforcement can directly strengthen responses which precede it by a time interval of more than a few seconds is contrary to the evidence" (p. 167). Second, Sommerhoff seems for the most part to be unaware of recent psychological work and theorising. This vitiates his account of language and animal behaviour, whilst enabling him to set up as Aunt Sallies Hull and Tolman who, except from the view point of the historian, no longer merit serious discussion. Third, the book contains a number of errors in logic. For example, Sommerhoff argues that pattern recognition is based on eye movements: he claims (p. 272) that it is easier to translate the extent and direction of an eye movement into a directional vector than to translate a retinal distance: he forgets that, since saccades are ballistic movements, the brain must compute visual angle and direction from the retinal image before initiating the eye movement.

Finally, and most importantly, despite the block diagrams and mathematical flourishes, the concepts Sommerhoff himself introduces do not seem to provide any new explanations of known phenomena and are less powerful than the concepts and explanatory models in current use by workers in the field. Thus, his classificatory and learning networks are not powerful enough to cope with what is known about the visual perception of objects, and his introduction of the notion of 'expectations' in this context remains vague. On the other hand, he is clearly aware that perception does involve the problem of inference and the construction and accessing of representational models, but he does not refer the reader to any of the extensive work that has been undertaken on such problems over the last decade.

The basic neural networks suggested by Sommerhoff are simple, but they do have a certain elegance. For example, he shows how, with the use of recurrent or forward inhibition, it is possible to construct networks that have the properties of flip-flops. The difficulties begin when he tries to explain complex behaviour or information processing by making more elaborate models in which his simple networks are put together into complex structures. At this point, the models become far from clear, and it is impossible to know whether they would behave in the way that is claimed or not: the only way to find out would be to simulate the models on a computer but Sommerhoff has not attempted to do this. Indeed, he dismisses the analogy between brains and computers in a few pages early in the book. He points out several differences between the hardware of existing

computers and that of the brain, but this is not really what is at issue, since computers are so constructed that they can be programmed to model any logical system within the limits imposed by their storage capacity and processing time. Having in this way foresworn computer simulation, he leaves himself without any good tool for constructing and testing models of the processes that underlie behaviour.

Throughout the second part of the book, Sommerhoff makes a valiant attempt to tie up function with brain anatomy. He assigns more or less plausible roles to some of the known connections in the brain, but in terms of the complexity of the tissue and of the behaviour which is controlled the assigned functions tend to be rather simple. The relationship between the evidence and the theorising is, as he himself admits, frequently tenuous.

The book is well written, and should bring a glow of nostalgia to those who pine for the slap-happy fifties when we could raise two cheers for anyone bold enough to tell us how behaviour could be taken care of by some neural nets and a few connecting tracts.

N. S. Sutherland

#### Scientific rambles

Science and Values: Patterns of Tradition and Change. Edited by A. Thackeray and E. Mendelsohn. Pp. viii+251. (Humanities Press: New York, 1974.) \$11.00.

In spite of its rather grand title, this collection of essays is a useful example of some pioneering work in the history of science. A more accurate title would have been "Science (or better, Scientists) in Social Context"; or even, considering parts of the text "What Some People Can Get Up To With Science". The editors disclaim any intention to air comprehensive or synthetic views: what they present are papers exploring strongly "contextual" approaches to the history of science from Rome to Tokyo, and from Manchester to Madagascar. This miscellany is the product of the Van Leer Jerusalem Foundation Conference in 1970.

With such rapid enlargement of the horizons of historical research it becomes difficult to find a theme common to even a few of the essays. The volume starts with a brisk general survey of the changes in English science since the eighteenth century leisured gentleman gave way to the nineteenth century worried middle class. There is a blow by blow description of the "Ayrton incident" of 1872, when the absolute supremacy of the Hooker dynasty at Kew Gardens was challenged by the eccentric Ayrton.

then Commissioner of Works. Thanks to Ayrton's removal because of an unrelated embarrassment, the scientific establishment escaped the humiliation of being rendered publicly accountable for their stewardship of public funds.

The most sensitive piece of 'externalist' historical writing comes from the American Charles Rosenberg, who describes the strategies of agricultural researchers returning from Germany in the 1850s. Here, we find cultural context, administrative constraints, and personal self-estimation, woven together very beautifully.

But the reader is not permitted to tarry on these familiar scenes; the anthropology of science beckons. Early nineteenth century Madagascar, its rulers anxious for 'magicians' to provide the tools of power, attracted a mixture of industrial, political and clerical entrepreneurs. This early "Technical Assistance Programme" failed to Europeanise the culture, though in a half century it did cause its decay.

Further east yet, to Japan and China; a sociological consideration of western style academic politicking in Tokyo, and the study of propaganda by a group of 'scientising' Chinese, who for a time after the First World War saw this as the truly modern alternative to Confucianism. With a sigh of relief we are brought back to the Europe of the 1920s, and a little tour of the great centres of theoretical physics. As a conclusion there is a highly theoretical essay on "The Authority of Science in Politics", which argues the implications of the presence of two discrete audiences, scientific and lay, with different criteria and concerns.

Does this amount to anything more than a record of a pioneering effort in a field which is no longer quite so pioneering? It certainly provides a warning that 'the social context' of science is vast and various and that scholars will need a strong hold on their material if it is not to baffle researchers and students.

J. R. Ravetz

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#### Politics and changes in the weather

Weather Modification in the Public Interest. By R. G. Fleagle, J. A. Crutchfield, R. Johnson and M. F. Abdo. Pp. ix + 88. (University of Washington: Seattle and London, July 1974.) £2.85; \$5.95.

Any good work of science fiction depends on one or two critical, and possibly fantastic, assumptions from which all the rest follows logically. In a similar way this study by Fleagle and his co-authors depends on the assumption that weather modification will become posible on a substantial scale, with reasonably predictable outcomes. Given this, the book discusses logically the political, legal, administrative and management structure which will be needed to control weather modification.

Some weather modification has already been demonstrated as practical—the clearing of cold fogs, for example—but that has raised few administrative problems. The seeding of orographic clouds to increase the snow and rain over mountains has also been practised for many years, apparently with some success, but that has not raised serious legal problems. If, however, a government agency could modify or divert hurricanes, or if it initiated cloud seeding on a grand scale then no doubt greater problems would arise.

There is a danger that the reader of this book who lacks a strong meteorological background will be led to believe that the potential of weather modification is much greater than has so far been proved, which may lead him, or her, to accept as necesary the somewhat elaborate organisation recommended in the book.

In the few legal actions brought in the United States under the quite extensive legal framework already established, none of the plaintiffs has been able to establish a cause-effect relationship between the actions of the weather modifier and the alleged results. This points to the premature nature of any elaborate legal or administrative framework.

On the other hand, the well-briefed meteorologist will find much of interest in the history of weather modification in the United States and in the views of the authors on future developments.

Whether or not one accepts the view that weather modification will be used on a wide scale in the future, an activity which currently involves the expenditure of around \$20 million of public funds in the United States certainly deserves public discussion. Furthermore, it is well to recall also that almost \$3 million is spent annually on cloud seeding by American companies operating abroad. The international aspects cannot be ignored: the effects may be greater on international relations than on climate.

John Sawyer

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#### Post script

The Freud/Jung Letters. Edited by William McGuire. Pp. xlii+650. (Hogarth Press, and Routledge and Kegan Paul: London, 1974.) £7.95.

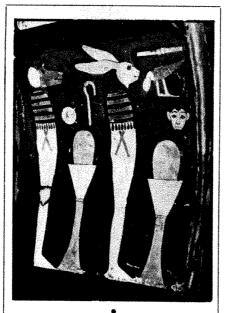
THE publication of this volume marks an important contribution to the history of the psychoanalytical movement, comparable to the issuing, in 1954, of Freud's letters to Wilhelm Fliess. Following his communications with Fliess. this exchange of material with his later protagonist, Jung, formed another chapter in the development of Freud's ideas. It is, perhaps, as the chronicle of a fruitful but doomed relationship that the volume has its greatest appeal, and as such, it forms a useful pendant to the official versions which have been given by Ernest Jones and by Jung himself.

When reading the letters, one is struck by the extent to which differences were managed for such a long time. For those familiar with the account of the break as given in *Memories, Dreams*, and *Reflections*, the tolerance demonstrated by Freud in the last years is unexpected. Yet the need felt by both men explains the concessions, the reconciliations, even the tensions that led to the final parting. A letter sent by Freud in 1907 sums up the situation beautifully:

. now of all times I wish I were with you, taking pleasure in no longer being alone and, if you are in need of encouragement, telling you about my years of honourable but painful solitude . about the indifference and incomprehension of my closest friends, about the terrifying moments when I myself thought I had gone . . about my slowly growing conviction, which fastened itself to the interpretation of dreams as to a rock in a stormy sea, and about the serene certainty which finally took possession of me and bade me wait until a voice from the unknown multitude should answer mine. That voice was yours" (p. 82).

Amid the intellectual torpor of the Burghölzi Hospital, Jung responded in kind. The meeting of two such gifted ninds inevitably acquired complicaions, for as Jung looked to Freud in erms of a father figure, so too the older nan regarded his younger colleague as i son and heir. Freud wanted Jung to explore schizophrenia in the way that ie himself had studied obsessional neuosis (p. 168). But even though Jung and begun to do that before the corressondence began, his reservations about in exclusively sexual origin for mental Ilnesses were evident from the first pp. 4-5, 7, 79, and 138-139). Later, as ung developed his own theories about he mind, disagreements became more narked. After Jung's lectures in America in 1912, an open break was invitable and it came within a year.

Publications and personalities fill



Hare-headed divinity of ancient Egypt on the coffin of Bakenmut, 21st dynasty. From *The Leaping Hare*. By G. E. Evans and D. Thomson. Pp. 262+24 photographs. (Faber: London, October 1974.) Paper, £1.50.

many of the letters, but the editor William McGuire aids the reader with footnotes which are concise as well as informative. It is a well produced book with lucid translations by Ralph Manheim and R. F. C. Hull, useful appendices, and an index. Finally, thanks should be given to the estates of both men for allowing the publication earlier than had been expected.

B. A. Boucher

#### Fifty year's minutiae

Half a Century of Medical Research. Vol. 1: Origins and Policy of the Medical Research Council (UK). A. Landsborough Thomson. Pp. xiv+308. (HMSO: London, June 1974.) £4,60.

WORTHY, rather than exciting, is the appropriate adjective for this book. It is the first of two volumes which will deal with fifty years of medical research in England. This first volume covers the history of the Medical Research Council and articulates the earlier principles of policy insofar as the future administration of the council and the future developments in scientific programmes are concerned. What comes out of this book—and, one can confidently expect, from the volume to follow-is almost every kind of fact and statistic that anyone interested in such matters could possibly hope for. Do you wish to know the details of the relevant Ministry of Health Act of 1919? Then this book will tell you just how this act was passed and how a very necessary legislative pro-

vision was inserted to cover the question of "The organization of enquiry and research on health activities". Do you wish to know the minutiae of the administrative organisation of the Medical Research Council: which subcommittee was formed to do this or that; that the title Second Secretary, first used in 1949-57 and revived in 1965, is now established for an officer able to act as the Secretary's secondin-command and deputy; that in 1914 rooms for temporary offices were taken at St. Stephen's House, Westminster, at a rent of £25.00 per annum, and that the council moved to Park Crescent in September 1961, into a building with a frontage of 255 feet with a depth of just over 42 feet? The book abounds in items like these.

As a living history I have to confess I find it lamentable, though as a compendium I find it invaluable. Nothing of the vibrating personalities emerges at all, nothing of people's real feelings, nor any real analysis of the infinite complexity of issues. So much is written up as a scientific Jennifer's Diary. We are told, for example, that on the occasion of the council's Jubilee Dinner, celebrated in November 1963, the Duke of Edinburgh was prevented from attending but that Lord Shawcross presided. In the absence of the Prince, The Right Honorable Quintin Hogg proposed the toast and a reply was made by Sir Henry Dale, but nothing of what was actually said on this occasion is recorded. Perhaps no-one really said anything-perhaps no-one knows; but I can hardly believe that. To add that: "The presence of Lady Fletcher and Lady Mellanby at the high table was particularly appropriate", may add something to our edification but nothing to our education.

This book is terribly safe; it says nothing controversial. There is very little that one can object to except that it is dull. Only a few notes of human vitality and spark emerge from the thumping melodic line. So, like drops of water in a desert, we read that the secretary of the council on February 2, 1939 wrote: "The discussion then wandered from the point and as we were both tired of cursing each other. we satisfied our minds by cursing everybody else". It is good to know that someone has a sense of humour. This is a book for the library but, unless you suffer from insomnia, it is not for the bedside.

I think it is a great pity that the sponsors have not been better served. The Medical Research Council, in achievements and policy, is an institution that we can contemplate with warmth and pride, and its history deserves a more vigorous and dynamic treatment.

June Goodfield

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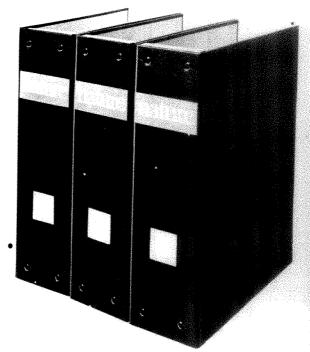
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# Astronomy for astrophysicists ...

Gamma-Ray Astrophysics. Edited by Floyd W. Stecker and Jacob I. Trombka. (A symposium held at NASA Goddard Space Flight Center, April, May 1973, sponsored by NASA and the American Physical Society.) Pp. xvi+412. (Scientific and Technical Information Office, NASA, Washington, DC, 1973.) \$2.90.

ASTRONOMY has been one of the most prolific of modern sciences, but the gestation period of one of its offspring  $-\gamma$ -ray astronomy—has been painfully long. The conception was in 1958 but it was not until 1972, after many false alarms, that birth finally occurred. This book is the report of a symposium on  $\gamma$ -ray astronomy which was held at the Goddard Space Flight Center in April 1973, and, therefore, marks a significant stage in the subject.

The papers read at the symposium illustrate very clearly the current situation in y-ray astronomy and the great differences between that subject and the neighbouring X-ray astronomy. X-ray astronomy began in 1962 with the unexpected discovery of an X-ray source outside the Solar System. This discovery was made with a detector of modest size on a short rocket flight; experimentalists immediately realised that, with the use of larger detectors and the much longer exposure times provided by satellites, a rich field was waiting to be exploited. By contrast, few results were forthcoming in γ-ray astronomy until the experimental techniques had been stretched almost to their limit. This aspect of the subject is illustrated by the experimental contributions to the symposium, many of which contain a great deal of discussion of the statistical significance of observations, and of the reality, or otherwise, of differences between experimental results.

Conference reports inevitably reflect the vices and virtues of conferences and this one is no exception. One of the worst aspects of conferences is the superficial manner in which each topic has to be treated because of the limitations of time; nearly all of the contributions to this symposium, both experimental and observational, have been treated much more fully in various journals. Many scientists believe that the real value of conferences are the discussions which take place outside the formal sessions. Obviously, this activity is not recorded in a report but something is retained in the reporting of the question time at the end of each session. Here, one finds a genuine element of criticism and discussion by Schwartz and Gursky of the mally in journals. A very valuable paper at the symposium was the discussion by Swartz and Gursky of the experimental problems encountered in measurements of the spectrum of the diffuse flux of cosmic γ rays. This feature was discovered as early as 1964 but the problems of eliminating the effects of atmospheric background radiation and the effects of cosmic ray interactions in the detector still have not been solved. A critical discussion of the experiments in this field was long overdue.

One of the breakthroughs in 1972 was the discovery by Chupp and his coworkers of y-ray spectral lines from a flare on the Sun. A paper by Ramaty, interpreting these results, illustrates the potential value of measurements of this type to our understanding of the role of high energy particles in flares. There is a contrast between this theoretical contribution and others which discuss subjects such as the decay of radioactive nuclei in supernova remnants, the interactions of cosmic rays in intergalactic space, and the possible effects of large scale regions of antimatter in the Universe. These discussions lack the discipline imposed by reliable experimental results and the more hardened experimentalists will look upon them with a degree of cyni-

The second breakthrough—the discovery of  $\gamma$ -ray bursts from outside the Solar System—was not published until after this symposium, but it was thought to be sufficiently important for two papers on the subject to be in-

cluded in the report.

The final session of the symposium was a discussion of future trends in γ-ray astronomy. This discussion leaves one with the impression that all γ-ray astronomers are aware of the difficulties facing the subject but that some, at least, are optimistic. The low price of this report, its clear format and its quick publication combine to make it an attractive summary of the current state of this branch of astronomy.

R. R. Hillier

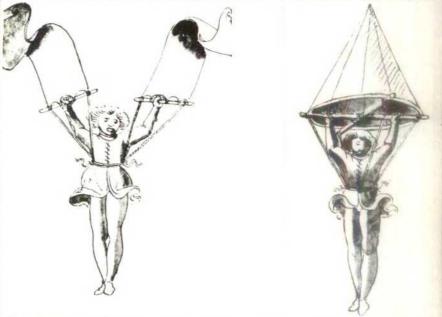
# ... and for arts students

Concepts of Contemporary Astronomy. By Paul W. Hodge. Pp. viii+547. (McGraw-Hill: New York, 1974.) \$9.95; £5.45.

Fundamental Astronomy, By Franklyn W. Cole. Pp. xiiiix+476. (Wiley: New York, 1974.) £5.30.

With deafening regularity new books on astronomy hit the librarians' tables, all aimed at that enormous amorphous bunch of students known as "nonscience majors" who annually flock to American universities to take degrees in liberal arts and letters. One gets the impression that no American publisher worth his salt can produce the yearly book list without at least one Introduction to Astronomy, Fundamental Astronomy, Modern Astronomy, Principles of Astronomy, New Horizons in Astronomy, Stars and Galaxies we Ought to Know and Love, Concepts of the Universe, The Universe and Beyond!, Mysteries of Space, and so on.

The teaching of science to arts students is obviously a good thing.



Parachutes: optimism and progress. Anonymous sketches from the late fifteenth century. The first recorded drop (probably an entirely appropiate word) was made in 1783. From Scientific Technology and Social Change. (Readings from Scientific American.) With introductions by Gene I. Rochlin. Pp. 403. (W. H. Freeman: SanFrancisco, 1974.) \$6.95.

Science impinges constantly on everyday life and ought to be understood at least in part. Astronomy is the most accessible science to the arts student: after all isn't every thinking person a cosmologist? Even the child ponders earth, space and time, their beginning and end. And astronomy is central among the sciences, gathering knowledge and expertise from a myriad of scientific specialities. Both the significance and insignificance of man in relationship to time and the universe are clearly illustrated.

But how should the arts student be approached? Most of the books seem to regard him as a rather stupid, inumerate, failed scientist. Surely this student has a different outlook on life from scientists, an outlook bred on comparison and criticism and aesthetic judgement, a preoccupation with why as opposed to the narrower scientific how. Science should be able to learn as much from arts students as they do from scientists, and these books should help and encourage arts students to play their part in quenching the thirst for knowledge.

Both the books under review are good in their own way. Both are clearly written in a concise and everquestioning style, continually leading the reader to grasp the enormities of the problems being tackled by astronomers and the many pitfalls on the way to understanding. Both books are lavishly illustrated with photographs of instruments, planets, stars and galaxies and, especially in Hodge's book, with thoughtfully designed simple line figures.

Each book considers basic astronomical concepts, the Solar System, stars and galaxies; however, Cole has slightly more emphasis on the Solar System, Hodge emphasising stellar evolution. Both books stress observation: Cole has an excellent appendix on observational astronomy for beginners; Hodge concludes each chapter with a fascinating hist of suggestions for simple experiments and observations. Some of these require simple laboratory equipment or a small telescope or high powered binoculars but many require only impoverished equipment that can be put together at home.

Choosing between the two books I would pick Hodge's Concepts of Contemporary Astronomy: the best book on astronomy at this level that I have read for many years. Hodge has thought very carefully about the needs of the students he is writing for and gives hem not only a knowledge of the nature of the universe but also an appreciation of the methods of science. But I still have a hankering to read an istronomy text written by an arts graduate. I wonder what they really make of it. David W. Hughes

#### Sources of streams

Fluvial Processes in Instrumented Watersheds: Studies of Small Watersheds in the British Isles. By K. J. Gregory and D. E. Walling. Pp. iii + 194. (Institute of British Geographers Special Publication No 6.) (Institute of British Geographers: London, February 1974.) £4.25; \$10.60.

THIS latest volume by the British Geomorphological Research Group reports the results of major process investiga- rather than competitive with, that tions in small catchments in Britain. The work involves contributions in four major areas: the origins and controls of run-off and of sediment and solutes. The objectives of the studies range from basic assessments of the volume and frequency of inputs and outputs to the basin, to formal testing of deductive models. For the most part, however, the work is empirical and much of it applied or capable of direct application. It should attract the attention of practising civil engineers as well as researchers.

Two dilemmas are brought out

clearly in the volume and referred to by the editors. The first is that within the basin there are important spatial and temporal variations in processes whose effects are integrated in sediment, solute and water yield at the output. Unfortunately, the output is where the monitoring of the basin is likely to be easiest and least expensive. In choosing small basins where one process tends to dominate, this dilemma has been partly avoided. Moreover, the work then becomes complementary to. carried out by other research organisations. The second dilemma is that with the rapid development of the subject, model building alternately precedes and succeeds data collection for testing. The solution seems to be the closer integration of the two areas of research.

This is an important addition to research in geomorphology and perhaps to hydrology. Despite an over-imaginative use of the linear regression model, it indicates a very healthy state in a key area of concern in environmental research and management.

John B. Thornes

#### Earth science by numbers

Geomathematics: Mathematical Background and Geo-Science Applications. By F. P. Agterberg. Pp. xvi+596. (Developments in Geomathematics, vol. 1.) (Elsevier: Amsterdam, London and New York, 1974.) Dfl135; \$54.00

This book surveys the mathematical methods and statistical techniques used in the study of data obtained from various types of physical and chemical sampling of rock and rock strata at the Earth's surface. It is not, as its title might imply, a comprehensive survey of the mathematical methods used in general geophysics. Rock samples, and geological data generally, are characterised by very great variability and so it is not surprising that this book concentrates on probability and statistics.

The book is unique in that these statistical methods are illustrated by many examples drawn from geology. In a way it is strange that it was biology rather than geology that provided the stage for the development of modern statistical methods. Lyell had in an appendix to his Principles of Geology divided the Tertiary into four periods on the basis of the proportion of species found in these rocks living today. But on the whole the field geologist, unlike the agriculturalist, did not find it easy to record in a simple numerical form the various pieces of information he collected and perhaps remained unconvinced of the value of doing so. It was of course the long series of carefully

marshalled numerical data at Rothamstead that gave the late Sir Ronald Fisher the impetus he needed to lay the basis of modern statistics.

It is, however, interesting to note that the impetus from the development of a statistical theory of variation in two and three dimensions resulted from the accumulation of data on the distribution of cross bedding directions and palaeomagnetic data respectively. All this is now changed and this book will be of great value to the geologist.

S. K. Runcorn

Statistical Methods for the Earth Scientist. By Roger Till. Pp. 154. (Macmillan: London and Basingstoke, 1974.) £5.00, boards; £2.50, paper.

THERE are few really readable books on statistics in general and even fewer aimed specifically at the earth scientist, but Dr Till is to be congratulated in joining a select and much needed band. The book is intended as the basis for a 40 hour course in statistics for undergraduate or postgraduate students with no previous background in statistics, and only simple algebra. It is a small volume-9.5 by 6 inches-with a content ranging from the basic philosophy of statistics up to analyses of variance, regression and some non-parametric tests. These are covered in a comprehensible way with examples drawn from mainly sedimentological and geochemical sources, although care has obviously been taken to include at least

one example from each of the main branches of earth science.

The book should form an excellent basis for a very elementary course (even less than the prescribed 40 hours) as it not only covers the basic concepts but also gives ample warnings of the standard pitfalls into which so many senior earth scientists have fallen—and are still falling. To quote one example, not used in the book, radioactive isotopic dates invariably include their precision, that is, the repeatability, yet most earth scientists still take these figures as measures of accuracy, at least until they conflict with their own ideas.

Other credits are good references and the avoidance of that frustrating phrase "it can be shown that"; instead Dr Till refers the reader elsewhere, usually to Yule and Kendall, for a more rigorous explanation but even that is rare. The publishers also deserve credit as the book is well produced and designed, although certain figures (for instance, 5.5, 5.6 and 5.7) are really too big and, if reduced, would not only have saved paper but would also have improved the appearance of the book.

The index covers only statistical subjects, and it might sometimes be necessary to find out where porosity or grain size is considered. But the only real fault is the scope: the range of analyses covered is much too short for the cost of the book and it would also have been a better buy if more statistical tables had been included.

I hope that Dr Till will be encouraged to expand this introductory text to include, for example, much fuller consideration of directional properties so that this book, if still sold at the same price, could become an essential text at the start of any earth scientist's career. But at the moment it does not go far enough.

D. H. Tarling

#### Model methods

Analytical Methods in Planetary Boundary-Layer Modelling. By R. A. Brown. Pp. xii+148+5 plates. (Adam Hillger: London, June 1974.) £8.00.

THE scope of this book is narrower than one might guess from the title. It deals primarily with a restricted topic in the theory of the Earth's atmospheric boundary layer, namely the mean velocity distribution in stationary, horizontally homogeneous, and neutrally stratified conditions. Particular emphasis is placed on the matching of an upper Ekman layer with a surface layer unaffected by the Earth's rotation. Concluding chapters consider the effects of non-neutral stratification and non-stationarity, but the treatment is very superficial. There are also two chapters on the instability of Ekman layers and the relationship between that and meteorological observations; this

is the best part of the book but its connection with the rest is not made clear.

For whom is this book intended? The author offers no indication, and the level of difficulty is so variable that one cannot guess. Who is the mysterious reader who can comprehend the equations of fluid motion with no derivation and little explanation, but who requires an elementary explanation of the procedures of dimensional analysis; who is already familiar with the properties of geostrophic flow, but not with the basic derivation of centripetal and Coriolis accelerations; who knows what is meant by "the eddy flux of potential heat", but who can usefully read a most superficial summary of the properties of thermal convection?

Meteorology students might benefit from a text leading up to current views of the atmospheric boundary layer through explanation of the now classical topics of Ekman layer theory and micrometeorology; synthesis of those topics is not so commonly discussed. Brown's book is, however, neither comprehensive enough nor lucid enough to serve that purpose. The derivation of the well known logarithmic profile, for instance, would be quite incomprehensible to anyone not already familiar with it.

Errors in the equations and algebraic expressions are sufficiently numerous to provide further reason for not recommending the book to readers unfamiliar with the subject. Most of the errors are probably misprints (although there are too many of them for this to be acceptable as an excuse). But there are incorrect signs on pages 49 and 79 which invalidate the subsequent algebra; and equation (3.19) is, I suspect, a misprinted version of an incorrect equation.

Research workers in fluid dynamics and meteorology may occasionally find the book a useful source on those topics for which the information is not gathered together elsewhere. But this purpose could have been fulfilled more effectively and economically by a review article on, for example, 'Ekman' layers in meteorology'.

D. J. Tritton

#### There's still no place like Holmes

Earth. (A series of books in Geology.) By F. Press and R. Siever. Pp. xi+945. (W. H. Freeman: San Francisco, July 1974.) £6.60.

THE authors of this book have impeccable qualifications for writing it, both as active research scientists and as university teachers. They say that it is intended for students who have had no previous college science courses; and they express the hope that it is as up to date as a current meeting of the Geological Society of America (it was finished in December 1973) and that at the same time, it is as understandable as today's newspaper. These intentions are commendable and the task they have set themselves is difficult; it is interesting to see how they tackle it.

The level throughout the book, which is splendidly produced and illustrated, is such that after reading it the diligent student should experience no difficulty with specialist papers in Scientific American. Plate tectonics figures large and early. The first 150 pages-"The earth as an historically evolved body and how we study it"-serve to introduce the vocabulary. They start with an account of the origin of the Solar System, which I found interesting because I knew nothing about it, and finish with a long chapter on rocks and minerals. Part II covers the traditional field of physical geology. Part III introduces the fields of petrology and geophysics and this section is written at a marginally higher level than the others; for example, a chapter on the formation of igneous rocks touches on Bowen's reaction series and on the problem of the origin of granite batholiths. I wonder, however, whether the 27 pages on plutonism and metamorphism really equip the reader to dip into F. J. Turner's book *Metamorphic Petrology* which is quoted in the bibliography.

How, then, does the book live up to its authors' intentions? Trying to cope with a readership which is assumed to be entirely without any scientific education has made the book unmanageably long. Was it necessary to devote three pages (115-118) to defining words like topography and contour? The need to start the chapter on weathering with the very beginning of inorganic chemistry makes it impossible to discuss the peculiarities of the weathering of rocks at all. The desire to encompass pass present and future in one large sweep reminds me of the phrenetic enthusiasm of geophysicists running an open day in the laboratory. For example, in chapter 3 ("How we find out about the earth") we have a photograph of a Benioff strain seismometer, a section on experimental petrology and a 'box' about Cavendish's 1798 determination of G, together with 13 other topics all introduced in the course of 22 pages. The book is certainly not dull, but I wonder whether the American college student has the stamina to read its 945 pages. I do know that the English girl studying for 'A' levels on whom I tried it, had not. In this field Holmes remains ancient but unbowed

D. H. Matthews

#### **Broad spectral lines**

Spectral Line Broadening by Plasmas. By Hans R. Griem. Pp. xiv+408. (Pure and Applied Physics: A Series of Monographs and Textbooks, vol 39.) (Academic: New York and London, May 1974.) \$31.50; £15.10.

An earlier book by this author, entitled 'Plasma Spectroscopy', has served for many years as a reference source for spectroscopic research in experimental plasma physics and astronomy. The popularity of that text, written in 1964, can be gauged by its high demand in departmental libraries, and the frequency with which it is encountered in spectroscopic laboratories. The new monograph treats a more specialised topic: spectral line-broadening mechanisms for neutral and ionised atoms in quiescent plasmas, and in nonequilibrium plasmas which exhibit suprathermal electric field fluctuations. It thus expands and updates chapter 4 of the earlier book, and similar theoretical reviews of the same vintage by Baranger, Van Regemorter, Traving and others

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static and impact approximations. Detailed examples of practical calculations using these two extreme, but complementary, approximations are then given; these are followed by a discussion of various intermediate approximations (phase integral, relaxation, dynamical and one-electron) for situations in which the frequency shift relative to the unperturbed line frequency is neither much less nor much greater than the characteristic frequency for single, binary, collisions with charged perturbers. Several other characteristic frequencies may enter the general problem. Thus, magnetic field and plasma correlation effects (Debye shielding, plasma polarisation shift and satellites caused by plasma wave fields) are selected and discussed in the final sections of a chapter which comprises some 40% of the total text. (In his preface the author wisely, though modestly, advises the experimentalist that a 'minimum theory' approach has been adopted.)

The next fifty or so pages constitute a highly selective, and therefore most interesting, critique of experiments which have provided important checks on overall accuracy, and validity limits to the various theoretical approximations. Most definitive experiments have been made on laboratory plasmas with electron densities of 1013-1019 cm $^{-3}$ , and temperatures of 2  $\times$  10 $^3$  - $2 \times 10^7$  K. This density regime is much more restricted than that of current practical and computational interest, which ranges from approximately 10<sup>3</sup> cm<sup>-3</sup> (H II region) to 10<sup>26</sup> cm<sup>-3</sup> (stellar interiors and laser fusion). Similarly, the available temperature range is still one or two decades less than that of thermonuclear interest. The experimenter should note how frequently density gradients have caused real complications in the interpretation of line shapes, even when some other, independent, density measure is also available. Relatively few line-broadening analyses seem to have been reported for heavy-ion hydrogenic lines and for lines in third and higher spectra, although plasma polarisation effects can be of particular significance to the latter spectra, because reliable corrections are needed to deduce the corresponding energy levels of isolated atom. At sufficiently high densities, neutral helium emits lines with forbidden components, involving transitions in which the angular quantum number does not change by unity.

More generally, the electric fields associated with suprathermal plasma waves will modify the line shape, and can excite satellites symmetrically disposed about the position of forbidden components. The third chapter, describing key experiments, therefore con-

cludes with a discussion of recent attempts to probe the intensity and orientation of turbulent electric fields, by measuring the intensity of sidebands on H<sub>B</sub> profiles in two orthogonal directions, and by analysing the polarisation of the emitted spectrum. Satellites on He I lines associated with electron and with ion plasma oscillations have also been identified; in some of these measurements wave energies three to four orders of magnitude above thermal levels were inferred. A particularly pretty example of the sophistication of present theory and experiment is illustrated in Fig. 28, in which Cooper and Hess have resolved fine structure caused by the Zeeman effect on a forbidden line, and on plasma satellites, by comparing  $\pi$  and  $\sigma$  polarisations

For the many of us who are users rather than specialists in the subject. the fourth and final chapter provides a very convenient summary and conclusion to the text. It outlines various practial applications, including determinations of plasma density and temperature, and the investigation of strongly excited collective modes in nonequilibrium plasmas; stellar emission, radio frequency lines from galactic regions, and the importance of the wing shapes of resonance lines to opacity and radiative transport calculations are briefly discussed. It also serves to introduce numerical data tabulated in some hundred pages of appendices. (Such information should be augmented in the future by infor mation collated at a data centre recently established at the National Bureau of Standards.)

One valuable service provided by a book of this sort is the correction of errors which have crept into the literature; such errors are discussed, for ex ample, at equations (207), (394), (395) and on pages 150, 176, 204 and 215 (references 145, 59, 258 and 38, respectively.) Presumably to avoid further errors, and to reduce costs, many of the tables are reproduced as computer printout; in the review copy some of these were difficult to decipher (for example, Table 3), but this constitutes only a minor criticism concerning the production of a book which is attractive to read, well organised and realistically priced. Professor Griem and his research school have made very significant contributions to this subject, and the book should be well received at a time when a topical interest in laser-fusion plasmas should give the subject a fresh lease of life. In this connection, it may be noted that the effects of extremely high densities, in which the plasma electrons obey Fermi-Dirac statistics, are not discussed explicitly.

Ian Spalding

Dr. H. MOENKE

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# Mathematical theory of elasticity

Introduction to Rational Elasticity. (Monographs and Textbooks on Mechanics of Solids and Fluids.) By C. C. Wang and C. Truesdell, Pp. xii+556. (Noordhoff: Leyden, 1973). Dfl. 130. FOLLOWING the precedent created by his joint work with Noll in presenting the non-linear field theories of mechanics Truesdell has now collaborated with C. C. Wang to bring together recent work in the mathematical theory of elasticity. As Truesdell points out in his introduction, there has been a dearth, until recently, of rigorous theoretical work on the existence and uniqueness of solutions to problems of elastic materials. With completely new techniques in pure mathematics becoming more generally understood, however, the stage is set for advances in many branches of applied mathematics. If this book inspires pure mathematicians to embark upon the application of rigorous mathematics to problems in applied mathematics and, in particular, elasticity then the authors' aims will have been vindicated and the colossal price of the book perhaps justified.

For those readers not familiar with the recent advances in pure mathematics, the book opens with a chapter which present the concept of a finite dimensional vector space, the concept of a manifold, and a section on Lie groups. This chapter is, as the authors admit, an outline of ideas, and many readers will need to study more deeply the topics covered before feeling confident to proceed further. Chapter II considers the more general subject of continuum mechanics, of which elasticity is one aspect. It is suggested that a reader whose interest lies solely in elasticity may ignore this chapter—that would be very unwise because in chapter II is the development of the ideas of constitutive relationships upon which modern elasticity theory is based. These relationships are further developed in the context of elasticity in the third chapter, which also contains a number of the inequalities satisfied by an elastic body, together with an introduction to the uniqueness and stability of solutions of elastic prob-

Homogeneous and inhomogeneous bodies are studied in the following two chapters, and chapter VI contains a discussion of wave propagation. In that chapter the less mathematical reader will recognise many familiar results and it could be argued that a good introduction to rational elasticity might be obtained by a superficial reading of chapters IV-VI before embarking upon a detailed study of these and

the earlier sections of the book.

The final chapter presents a unified account of the present state of knowledge of the existence, uniqueness and stability of the boundary-value problems of elasticity. This is the climax of the book for the mathematician and should leave him in no doubt that there are still many problems worthy of his skilled attention.

A. H. Craven

#### Crystal behaviour

Dislocations and Plastic Deformation. By I. Kovacs and L. Zsoldos. (International Series of Monographs in Natural Philosophy, Vol. 60.) Pp. xii+343. (Pergamon: Oxford and New York, February 1974.) £3.50.

This book aims to provide an introduction to dislocation theory, followed by an account of the mechanical properties of crystals, including the yield stress, work hardening, creep, and effects of heat treatment. It covers much the same ground as Cottrell's famous book Dislocations and Plastic Flow in Crystals, but attempts to update and enlarge upon that work, now 20 years old. It is a difficult task, for the boundary between dislocation theory and plasticity is as much of a no-man's land as ever, and Kovacs and Zsoldos can only list the conflicting theories of work hardening without leaving the reader much wiser; a student will find this chapter heavy and demoralising.

It is unfortunate that the treatment is so exclusively devoted to tensile stressstrain curves of pure face-centred cubic metal single crystals: a wider discussion of the experimental observations would be rewarding. Another general criticism of the book is that it omits many recent developments: there is no mention of Foreman and Makin's computer experiments on yielding, which have greatly clarified the subject; no mention of weak-beam measurements of stacking-fault energy and no mention of diffusion creep. Finally, the book contains a number of errors, perhaps the most glaring being the expression for the energy of a straight dislocation on page 48, which leads to erroneous expressions for the line tension on page 64, and this in turn, together with the omission of weak-beam results, leads to a rather unbalanced assessment of the current value of stacking-fault energies.

In spite of these defects, the book is more comprehensive and up to date than any now available, and provided the graduate student has read Cottrell's book referred to earlier, this book can be used as an introductory text in a field where few are available.

L. M. Brown

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dited by Allen I. Laskin PH.D. and Hubert echevalier PH.D. Condensed Edition, Decemer 1974, 944 pages, 150 illustrations. RC Press, £10.00.

#### **Blackwell Scientific Publications**

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#### Methods for cool operators

Low Temperature Laboratory Techniques: The Use of Liquid Helium in the Laboratory. Second edition. By A. C. Rose-Innes. Pp. 255. (English Universitiies: London, 1973.) £3.95.

This is a new edition, brought up to date (although no references are later than 1971) and expanded with sections on developments since 1964. The main sections are concerned with the temperature range above 1 K, with coverage of the handling of helium, cryostats, and the design of apparatus, and the measurement and control of temperature. Towards the end are chapters on the use of 3He (including dilution refrigeration, but not Pomeranchuk refrigeration) and on temperature measurement below 1 K.

The advice given is at an unusually detailed practical level, often to such an extent that every dimension and technique seems to carry a personal guarantee. The numerous figures reinforce this feeling by their directness and simplicity. I can see the value of this approach, particularly for the beginner who often has to take responsibility for the lowlier aspects of research without the author's experience. Inevitably, with such a laboratory-workbook format there are places where the reader may wish to add, remove or alter, but on the whole it is a useful collection of highly practical tips. The data tables are also helpful.

There are a number of unnecessary variations of notation (for example He<sup>3</sup> on p. 193; helium<sup>3</sup> on p. 194; ml on p. 22; cm3 on p. 23); misprints (I enjoyed "owl temperature" on p. 99 and "aneroid" on p. 114 among others) and unnumbered equations. Generally, though, appearance and visual clarity are good.

D. S. Betts

#### Nuclear structure

Theoretical Nuclear Physics. Vol. 1: Nuclear Structure. By Amos de Shalit and Herman Feshbach. Pp. xxviii+ 979. (Wiley: New York and London, June 1974.) £14.60.

THE appearance of this volume is a notable event for nuclear physicists and yet another reminder of the loss to the scientific community caused by the untimely death of Amos de Shalit. It is the first of two volumes covering essentially the whole of nuclear physics; this one is devoted to nuclear structure and a forthcoming volume will cover nuclear reactions

The authors aimed to cover all the important ideas as clearly as possible, and in a way that will inspire as well as instruct, and they have succeeded brilliantly. A brief summary of the contents will indicate the scope of this encyclopaedic work.

The first chapter is an introductory review of nuclear physics, dealing with nuclear sizes and masses, forces and energies, and also with nuclear systematics, regularities in nuclear spectra, electromagnetic properties of nuclei, high energy scattering and reactions and associated topics. Subsequent chapters are devoted to nuclear models, starting with the simplest that likens the nucleus to a Fermi gas. There are sections on the Weisäcker mass formula, one and two-particle densities, coulomb energies and the nucleonnucleon force and nuclear stability. Next comes a chapter on nuclear matter, basic to the understanding of nuclear properties. The independentparticle and independent-pair approximations are described, together with the Bethe-Goldstone equation and its solution.

Two chapters are devoted to the independent particle and shell models. It is shown how the single-particle shell model with either an harmonic oscillator potential or a more realistic rounded and finite potential is able, provided a spin-orbit term is included, to account for many basic features of nuclear structure, in particular the magic numbers and the spins, parities and magnetic moments of nuclear states. The model works best for nuclei near closed shells, but it is also shown how it may be extended to nuclei with several particles outside closed shells, and its success is illustrated by examples. The Hartree-Fock self-consistent field theory is developed and applied to closed and open-shell nuclei. The method of classifying nuclear states with the help of the concept of isospin is described and illustrated by examples, using various coupling schemes. There are also sections on particle-hole configurations, fractional parentage coefficients and configuration mixing in many-particle systems.

Away from closed shells it becomes increasingly difficult to use the shell model to describe nuclear states because of the very large numbers of possible configurations, and for such nuclei the collective models are often more appropriate. In such models many nucleons are assumed to move together, either as a rotation or as a vibration of the whole nucleus. With an appropriate collective Hamiltonian it is possible to calculate many of the properties of such nuclei.

The theory of multinucleon systems is considered next, with a detailed account of the Hartree-Fock theory, the random phase approximation and the free quasi-particle system, all using the second quantisation formalism. There are sections on the pairing interaction, the linked-cluster expansion, the Brueckner-Hartree-Fock theory, the local density approximation, the pairing-plus-quadrupole interaction and the microscopic theory of valence forces.

The last two chapters are devoted to electromagnetic transitions and weak interaction phenomena. After a description of the nuclear electromagnetic current and the quantised electromagnetic field the selection and sum rules governing electromagnetic transitions are obtained and illustrated by examples. The section on weak interaction begins with the theory of  $\beta$ decay and goes on to consider different types of transitions, orbital electron capture, antineutrino absorption, electron-neutrino angular correlation, the helicity of the neutrino, muon decay, vector currents, forbidden beta decay and absorption of muons by nuclei.

This brief summary of the main topics can do little to indicate the wealth of detail and comprehensiveness of the coverage of the book. It is difficult to think of any important aspect of nuclear structure physics that has not been covered, and the vast range of topics is welded into a coherent whole.

The treatment is generally at the graduate student level, and requires only a general familiarity with nuclear physics such as may be obtained in an undergraduate course. It will also serve as a valuable reference book for undergraduates and a handbook for professional nuclear physicists.

P. E. Hodgson

# Denial of quantum physics

The Interpretation of Quantum Mechanics. By Michael Audi. Pp. xiv+200. (University of Chicago: Chicago and London, 1973.) £5.75.

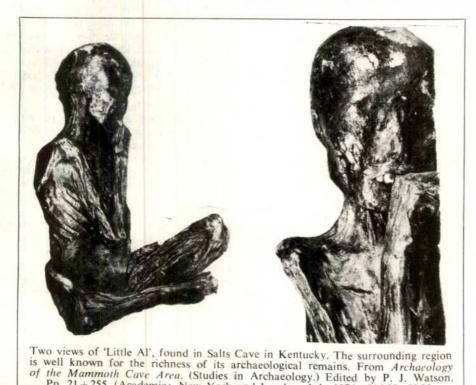
THE main purpose of this book is to show that the classical conception of a particle, particularly the attribution of simultaneous position and momentum to a particle, can be carried over to quantum mechanics. The core of the argumentation runs as follows: classical mechanics can be separated into two parts. First, a material body can be represented as a coffection of particles, each particle having at all times an exact position and an exact momentum and thus travelling in a continuous path in space. Second, the path of a particle is determined by laws of motion. In quantum mechanics, however, there exist no laws of motion for atomic particles, and the indeterminacy of observations is due to the unpredictability of the paths of material atomic particles.

This means, of course, that it is erroneous to ascribe any wave property to a material atomic particle. Conversely, the particle property of photons is denied. A photon is considered to be oscillations in the electromagnetic field, and all the experimental evidence of the corpuscular character of electromagnetic radiation is explained as "discrete interaction properties" of the electromagnetic field with matter. Obviously this amounts to a

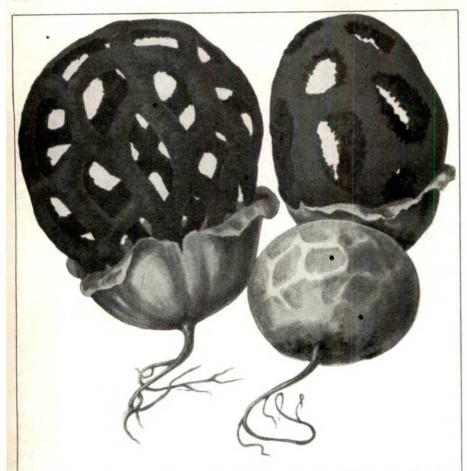
total rejection of the complementari

By means of his thesis about th unpredictability of the (postulate paths of material atomic particles, th author is to some extent capable protecting his classical conception an atomic particle from the exper mental facts in quantum physics. Br on page 106 it is rightly stated the satisfactory interpretation quantum mechanics must provide a explanation of crystal diffraction phenomena. Now the classical partic concept will come to its crucial tes the reader thinks. But instead of intre ducing in some statistical way th postulated particle trajectories, the it cident beam of particles is represente in the usual quantum mechanical wa by a plane wave function, which then perturbed by a periodic potentia representing the crystal. Though it indeed an interference phenomeno which the author sketches in the calculations, he does not like to sa so. Multiple slit experiments are treate along the same line, and the argumer ends up with the rather cryptic state ment that each particle in the bear passes through one particular slit, bu nevertheless, more than one slit is ac ing on the particle.

The parts of the mathematica scheme of quantum theory involve in the argument are far too sketchil presented and several equations at blurred by misprints. Also, the author seems to minimise the significance ( points which oppose his basic position and in some cases he simply leaves or such points. For instance, the Coper hagen interpretation of quantaum mech anics is said to be inconsistent because it claims that quantum physics contain classical physics as a limiting case an yet it does not consider atomic particle as classical particles. One should expec then, that the author would examin very carefully the correspondence be tween the two fields of experience. O pages 34-35 he claims to have skatche a derivation of the classical Hamilton Jacobi equation from Schrödinger equation in the limit where the quantur of action can be considered to b vanishingly small. It really cannot b called a derivation because it involve equations which are mathematically ob jectionable, and, furthermore, the mos important result in this connection namely that the wave packet repre senting the atomic object moves accord ing to the laws of classical mechanics is left out. Yet this result shows that the complementary behaviour of atomi objects displayed through interference phenomena and particle phenomena fully compatible with the classical par ticle concept, applying, of course, onl to observational situations where th quantum of action can be neglected.



Pp. 21+255. (Academic: New York and London, July 1974.) \$16.00; £8.00.



Clathrus cancellatus. One of hundreds of illustrations in Mushrooms and other Fungi. By Augusto Rinaldi and Vassili Tyndalo. (Translation by Italia and Alberto Mancinelli.) Pp. 333. (Hamlyn: London and New York, November 1974.) £6.95.

The author's thesis about atomic particles means, in fact, the rejection of quantum physics itself. If the reciprocal limitations in the applicability of the space-time concepts and the momentum-energy concepts expressed by Heisenberg's indeterminacy relations are not fundamental, but can eventually be overcome by improvements in observational techniques and theoretical methods, then there exists no universal quantum of action and no quantum physics.

T. Bergstein

#### **Molecular chemistry**

nternal Rotation in Molecules. Edited by W. J. Orville-Thomas, Pp. xvii+606. (Wiley-Interscience: New York, 1974.) £13.50.

The last thirty years have seen considerable activity in the study of internal rotation and related ring puckering motions in molecules. Or-ille-Thomas' extensive volume is timely, containing a chapter on all of the main techniques and the results which can thus be obtained. It is, unfortunately, an edited volume with eparate authors for each chapter, a scheme which leaves a few gaps and inroduces some duplication and variation of style. It is a pity that the editor

did not impose a *fiat* on the question of units for barrier heights: the choice of kcalorie mol<sup>-1</sup> dominates over the more favoured kJ mol<sup>-1</sup> by about 2: 1. It is perhaps inevitable, but many of the chapters take space to introduce fundamentals, so that the volume includes unnecessary introductory accounts of nuclear magnetic resonance (n.m.r.), gas microwaves, electron diffraction and acoustics.

Gas microwaves provide the single most valuable technique for gaining information about the gas phase and the topic is covered by N. L. Owen in a fairly short but effective chapter. Electron diffraction, described by A. H. Clark, is valuable chiefly for indentifying the dominant conformation, and in some cases for evaluating the trans-gauche ratios. Acoustic work, described by S. M. Walker, covers the kinetics of the transformation, although the size of dispersion also indicates energy and volume differences between the interconverting systems. There is also a helpful chapter on theoretical work, covered by A. Veillard, from which it is clear that elaborate ab initio calculations can evaluate the barriers effectively although it is not easy to extract simple qualitative information about the origin of the

# Gradient liquid chromatography

Gradient Liquid Chromatography. By C. Liteanu and S. Gogan. (Elli Horwood Series in Analytical Chemistry). Pp. xii+358. (Ellis Horwood; Chichester, Distributed by John Wiley. Halsted Press: New York, July 1974.) £10.50.

This work reviews the principles, practice and application of liquid chromatography using various types of gradients in eluent and stationary phase composition, and in temperature. The review is comprehensive up to 1969 or 1970 but the authors have unfortunately failed to react fully to the recent development of high performance liquid column chromatography and the practical parts of the book are somewhat dated although still useful as a basis for development.

In reviewing the theory the authors are too uncritical and by quoting numerous mathematical results, often without adequate explanation, they have failed to explain the principles of the subject as clearly as they could have. This is unfortunate in an otherwise useful work for it limits its value to practising analytical chemists who badly need a clear and simple exposition of this important topic.

John H. Knox

barrier from such complex wave functions. Simpler functions are ineffective in predicting the barrier heights.

One of the difficulties in this field is the major influence of solvents, and in liquid phases the effective barriers and potential minima may be quite different, a point strongly emphasised by R. J. Abraham and E. Breitschneider. The regular tools for liquid-state studies include n.m.r. and dielectric methods.

Other chapters complete the picture which, overall, amounts to a great deal of work by many research workers. There is sufficient knowledge to allow most unknown barriers to be inferred by analogy and interpolation, at least in the organic field. References to completely inorganic molecules are very sparse and there did not seem to be any reference to the intriguing motion which makes all the fluorine atoms indistinguishable in PF<sub>5</sub> at higher temperatures. A formulae index would have added greatly to the usefulness of the book.

Clearly, most specialist science libraries will need this volume, but the overall picture is neither sufficiently clear nor sufficiently intriguing to warrant individual purchase.

D. H. Whiffen

#### **Connective issues**

The structure and Biochemistry of Cartilage. By A. Serafini-Fracassini, and J. W. Smith. Pp. vii+236. (Churchill Livingston: Edinburgh and London, 1974.) £8.00.

This book is a very readable account of the biochemistry of connective tissue components, and of the structure and morphology of cartilage. It provides a good background to the biochemistry of connective tissue and gives an excellent introduction to cartilage morphology with particularly detailed accounts of the epiphysial plate and calcification. In those topics it is a major advance upon existing texts.

The first half of the book gives a detailed account of the structure of elastin, collagen and the glycosamino-glycans. The subject is treated thoroughly and would do justice to a more general text on connective tissue biochemistry. Much of the information is contained in numerous tables which compare chemical compositions and amino acid analyses, and list physicochemical properties. This concise way of presenting data is backed by a text that is informative and to the point, which greatly enhances the value of the book to the general reader.

The molecular biology of connective tissue macromolecules is probably the most rapidly developing field of research relating to cartilage, and the inevitable delay between the finish of literature searches and publication has produced some points at which these sections are now out of date. For example, there is little emphasis on the different genetic species of collagen present in different tissues. The distinction in composition between cartilage collagen [aI(II)]3 and skin-bone collagen  $[\alpha I(I)]_2$   $\alpha_2$  is described, but their significance as gene products that are tissue specific is not discussed at all. Similarly, new information on the organisation of proteoglycans has shown that they aggregate by binding to hyaluronic acid, forming very high molecular weight complexes within the cartilage matrix. The staining of proteoglycans in cartilage sections for electron microscopy has, therefore, more recently been reassessed taking this into account. The problem that information becomes rapidly out of date is always present to a greater or lesser extent in scientific reviews but in this instance it does not detract from the usefulness of the large amount of additional information presented.

The second half of the book is concerned with the morphology of different types of cartilage. The transition from biochemistry to morphology is bridged by two chapters in which tech-

niques of studying connective tissue components with both the optical microscope and electron microscope are appraised critically, and the general ultrastructure of cartilage is reviewed. The remainder of the book then deals in some depth with the development and structure of the epiphysial plate and with the calcification of cartilage. It finishes with shorter sections on articular cartilage and elastic cartilage. The chapters on the epiphysial plate are particularly worthy of mention as they give a comprehensive account of its development, structure, nutrition, the formation of matrix, and the presence of lysomal enzymes during the subsequent calcification of the tissue. It is difficult to find these topics described in such detail elsewhere.

The book, primarily a text on carti age, can perhaps be criticised for the allocation of excessive space to conne tive tissue biochemistry. Much of the research on the structure of elastin an collagen does not relate directly t cartilage. The book also lacks an attempt at a systematic account of th various types of mammalian cartilag or indeed any discussion of phylogen and for these reasons it cannot be re garded as a comprehensive work. Th general standard of presentation is however, very high. There is an abunc ance of illustrations and a particularl large number of electron micrograph: Each chapter also contains an exter sive bibliography which enhances it value as a reference work.

T. E. Hardinghar

#### Cholinergic transmission – from Russia

Acetylcholine: an Approach to the Molecular Mechanism of Action. By M. J. Michelson and E. V. Zeimal. Translated from the Russian by E. Lesser and Mira Lesser. (International Series of Monographs in Pure and Applied Biology. Division: Modern Trends in Physiological Sciences, vol. 38) Pp. xviii+241. (Pergamon: Oxford and New York, December 1973.) £8.50.

INTEREST in the application of techniques of molecular biology to the study of drug-receptor interactions, in particular at the cholinergic synapse, has grown rapidly in recent years. So the advent of a book claiming to discuss the "molecular mechanism of action of acetycholine" would seem to be of vital and topical concern. But the book fails to generate the interest promised by its title. That may be partly because the authors concentrate much of their attention on the more classical "chemical-pharmacological" approach of determining structure-activity relationships; but it may also reflect the fact that, despite the initial advance afforded by success in the isolation of the cholinergic receptor, further progress in elucidating the molecular events whereby acetylcholine produces its efforts has been disappointing slow.

Nevertheless, the book includes much material of interest to students of cholinergic transmission. The authors describe work carried out by themselves and a variety of colleagues in Russian laboratories over the past 30 years. The reference list shows that most of this work has been published only in Russian, and may therefore be new to English speaking readers.

There is an initial introductory chapter on the electrophysiological and biochemical functioning of the cholinergic synapse, with an interesting account of what seems to be the fire recording of the endplate potential, be Ginetsinsky and Michelson, in 193: There follows a brief but informative discussion on the current knowledge of the chemical structure of choosinoreceptors. Again it is pleasing the read of evidence obtained by Turpae in the early 1960s for the presence of sulphydryl groups in the acetylcholian receptor, which anticipated the experiments of Karlin and his colleagues.

Theories of drug-receptor interac tion are assessed well in the thir chapter but the mathematical trea ments are unnecessarily complicate by the avoidance of conventions symbols. The following three chapter deal essentially with results obtaine from comparing the activities of variety of acetylcholine analogues of both the acetylcholine receptor an acetylcholinesterase, from which th authors attempt to make deduction about the active centres of both mole cules, and about the arrangement of receptors (or subunits of the receptor on the postsynaptic membrane. Unfor tunately, the treatment is length? and repetitive and the derived information is scanty and, at best, equivocal.

The final chapter, on non-synapti cholinoreceptors, draws together dat from a variety of tissues includin feetal and denervated muscle, autone mic ganglia, Renshaw cells and Aplysi giant neurones, and fills in many 0 the gaps left in previous chapters. One again, there is a surprising reference to results obtained by Ginetsinsky an his colleagues on foetal muscle some years before similar observations wen made in laboratories outside Russia. I is to be hoped that publication of this book in English (an excellent transla tion) is a sign that such failures in communication will be less frequent Vasanta Srinivasai in the future.

# Primates: swinging through the trees . . .

Primate Locomotion. Edited by Farish A. Jenkins, jun. Pp. xii + 390. (Academic: New York and London, January 1974.) \$34.00; £16.30.

THE disadvantages of the current tendency to publish compilations of invited articles on specialised subjects are numerous. Though books of this kind have undoubted advantages, common to convenience foods and package holidays, they lack the combination of authoritative overview and synthesis of the single-author volume. One of their most serious weaknesses is that, being invited articles, they escape the wholly desirable quality control that anonymous referees and a critical editorial board can impose. Furthermore, the incautious reader may suffer in a different way when he discovers that only some of the articles are really new. Too often their substance, if not their dressing, has already been published elsewhere

The principal fault of *Primate Loco-motion* is the lack of overview and synthesis. With two or three exceptions the articles are new. They are generally of a high standard, lively, well presented and illustrated. It is apparent that currently at least two major problems are engaging the interest of

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workers in the fields of anatomy, primatology and primate palaeontology. One of these—the arboreal ancestry of primates—is long overdue for resurrection; the other—brachiation and human ancestry—is a prime candidate for interment.

Cartmill sets the scene for the former in his discussion of the relative adaptive values of the clawed paw and the prehensile hand for arboreal life. He asks fundamental questions and supplies convincing answers illustrated by a wealth of comparative data from other mammalian groups. Too little attention has been paid in the past, as in this otherwise excellent chapter, to the sensory aspects of hand function. hypothesis, of the primacy of arboreality in the evolution of monkeys and apes is incomplete without consideration of the role of the peripheral sense organs

Chapters from Farish Jenkins on former in his discussion of the relative locomotion of treeshrews, by Szalay and Decker on the evolution of the primate tarsus and by Walker on the locomotion of present day prosimians, pursue this theme. Jenkins's paper, as becomes an editor's contribution, is particularly sound and lacks any element of witch-hunting which diminishes the stature of some of the other articles. I particularly like Jenkins's final sentence which runs: "The adaptive innovation of ancestral primates was not the invasion of the arboreal habitat, but their successful restriction to it".

The brachiating theme is pursued in the chapters by Lewis, by Roberts on the structure and function of the scapula, and by Tuttle and Basmajian. O. J. Lewis states his views once again on hominoid wrist morphology which lead him to espouse Sir Arthur Keith's theory of the brachiating origin of man in spite of the fact that Keith was basing his views on the gibbon, which Lewis clearly rejects from human ancestry on the grounds of wrist morphology.

Tuttle and Basmajian, reporting on an electromyographic study of forearm muscles of the gorilla, give considerable space to the well aired subject of knuckle-walking in African apes. Their contribution to the brachiating issue is, however, both moderate and sensible

A valuable chapter on the mechanics of locomotion in primates by Badoux, an account of leaping in galagos by Jouffroy and Gasc and an interesting article on the much neglected field of postural activities in Old and New World monkeys by Michael Rose, complete the volume.

Primate Locomotion is an important source book for the graduate reader but the lack of overview and synthesis makes it an unsuitable text for the student.

J. R. Napier

# ... and adapting to other situations

The St Kitts Vervet. By Michael McGuire. (Contributions to Primatology. Vol. 1). Pp. xi+202. (S. Karger: Basel, London and New York, 1974.) Sfr 56; £8.10; \$17.40.

VERVET monkeys were introduced to St Kitts, probably from West Africa, in the latter part of the seventeenth century. Today, they are common both in scrub-covered coastal areas and in the forested ravines running up into the island's central massif, and population densities are similar to those observed in several African field studies. McGuire's monograph reports the results of 6,300 hours of observation by nine observers in nine study areas, and covers population demography, activity patterns, social organisation and communication. Two main findings emerge. First, groups living in the ravines differ in dispersion, activity patterns, and in the frequency of grooming and agonistic interactions from those living in the coastal scrub. Second, 19% of the (54) communicative gestures observed in East African vervets by Struhsaker and 44% of the (48) vocalisations were not recorded in the St Kitts population.

Both comparisons make an important contribution to our knowledge of the adaptability of primate behaviour. Unfortunately, they are spoiled by inadequate quantification. In the first, the reader is asked to take on trust many of the suggested differences and no quantitative evidence is either shown or referred to. Where this is not the case, description of sampling methodology and sample size is scanty and no attempt is made to test the significance of the observed differences. In several cases, the measures used are inappropriate: for example, the "general tension state" of each group was measured by estimating its flight distance from an observer. Comparison with Struhsaker's study is largely confined to qualitative differences and is free from these objections. But the reader is required to accept the author's opinion that the absence of the communication patterns observed Struhsaker is not the product of differences in classification rather than in behaviour.

A second major criticism of the monograph is the amount of turgid and unnecessary discussion which it contains. This suggests that, despite the impressive list of series editors on the front cover, little editorial attention was paid to the manuscript. As a result, material which would fill two medium length papers has expanded into a 200 page book.

T. H. Clutton-Brock



# INTERSCIENCE



#### **GENETICS AND THE ANIMAL CELL**

By M. Terzi, Imperial Cancer Research Fund, London.

Cell biology is now very much in vogue and recent techniques have now turned toward the mammalian cell. Large research programmes are concerned with the nature and treatment of cancer; others are concerned with the problems of ageing, human genetics or cellular aspects of development. Students and those involved in research in this "new biology" will want to read this book, for the fact is that more is known about organisms than about their cellular constituents. Dr. Terzi has gathered together a great deal of information about the mammalian cell regarded as a genetic system. He investigates the genetic structure of somatic cell populations and re-examines concepts such as cell senescence, transformation, modulation, differentiation and re-differentiation. He also has new things to say on chromosomal variation and the effects of variations in gene dosage on the behaviour of cell mutants.

October 1974

270 pages

£6.50

#### QUALITY CONTROL IN BLOOD BANKING

By Byron A. Myhre, M.D., Harbor General Hospital.

Illustrates attitudes and techniques for attaining and maintaining quality in blood banks and transfusion services (Quality Control Methods in the Clinical Laboratory).

October 1974

252 pages

£8.10

#### **GENETICS AND SOCIAL STRUCTURE: Mathematical** Structuralism in Population Genetics and Social Theory

Edited by Paul A. Ballonoff, University of Texas.

Contains papers on kinship and marriage considered from a social perspective as well as rare papers on population genetics. Includes the first published work of Charles Cotterman on the probability formulation of genetics and the first publication in English of the famous paper of Courrege on elementary structures of kinship. Editor's commentary provides additional references and historical material and expresses a philosophy that a unified theory is possible for the kinship structures of social and genetic theories. (Benchmark Papers in Genetics.)

September 1974

520 pages

£11.90

Published by Dowden, Hutchinson and Ross Inc., and distributed by John Wiley and Sons Ltd.

#### ESSENTIALS OF NEMATODOLOGY, Volume 8, Oxyuroidea By K. I. Skrjabin et al,

Helminthological Laboratory, Academy of Sciences, Moscow, U.S.S.R.

Volume 8 in this series treats the subfamily of oxyuroidea. The authors set themselves the objective of generalizing on the morphology, biology, ecology and geography of all the known members of this group of nematodes parasitizing vertebrate and invertebrate animals and man in all parts of the globe. Academician Skrjabin and his colleagues undertake an extensive review of these nematodes and propose a new system of determining all oxyurides. (Translated from the Russian.)

July 1974

500 pages

£18.75

Published by Israel Program for Scientific Translations Ltd., and distributed by John Wiley and Sons Ltd.

#### PHYSICS OF DROP FORMATION IN THE ATMOSPHERE

By Yu. S. Sedunov, Main Administration of the Hydrometeorological Service, Council of Ministers of the U.S.S.R.

Processes of drop assembly formation and condensation growth are considered. Phase conversions during formation and development of clouds are studied. The equation of the condensation growth of an individual drop is analyzed in detail. The Theory of condensation nuclei and the theory of an initial condensation stage are given.

Much attention is paid to the description of kinetic processes for cases when condensation is regular and when meteorological paramenters fluctuate. Fluctuations of these parameters in the atmosphere caused by turbulence are considered. (Translated from the Russian.)

September 1974

244 pages

Published by Israel Program for Scientific Translations Ltd., and distributed by John Wiley and Sons Ltd.

#### **PYRIDINE SUPPLEMENT IN FOUR PARTS, Part 3**

Edited by R. A. Abramovitch, University of Alabama,

Covers rapid growth in pyridine chemistry of the past ten years. The use of tables assists workers who wish to find at a glance whether a given reaction has been carried out or a given compound has been prepared and by what method. (Chemistry of Heterocyclic Compounds, Volume 14.)

October 1974

1268 pages

£36.00

#### ADVANCES IN SATELLITE METEOROLOGY, No. 2

Edited by N. K. Vinnichenko and A. G. Gorelik, Main Administration of the Hydrometeorological Service, Council of Ministers of the U.S.S.R. Central Aerological Observatory.

This collection contains theoretical and experimental papers devoted to the propagation of microwave and infrared radiation in the atmosphere. It is shown that the introduction of modern infrased and microwave instrumentation opens up new possibilities for airborne and ground-based measurements of a number of atmospheric parameters. The collection will interest scientists working on the development of radiophysical methods for atmospheric studies and specialists in physics of the atmosphere. (Translated from the Russian.)

September 1974

154 pages

77.20

Published by Israel Program for Scientific Translations Ltd., and distributed by John Wiley and Sons Ltd.

#### STUDIES IN CRYOBIOLOGY

By L. K. Lozina-Lozinskii, Institute of Cytology, U.S.S.R. Academy of Sciences.

The book deals with the adaptation and resistance of unicellular organisms, polkilothermic animals, and cells and tissues of animal origin to low and extremely low temperatures.

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Once upon a time young Englishmen nuclear magnetic resonance, and elec- has little relevance to the general study would make 'the grand tour' through tronic spectra and optical activity. This of biochemistry". the countries of Europe as finishing experimental emphasis on proteins touches to their educations. This book is makes it all the more surprising that largely concerned with cells and their just such a tour through selected areas no chapter has been dedicated com- components. The emphasis is still strucof biochemistry. Despite a cautiously pletely to protein structure and stabi- tural, but several contributions give deworded preface, it is unmistakably an lity. After all, equally important and tailed consideration to the use of intact audacious attempt to present, in one complex discussions are presented for and living cells as tools for the biochevolume, the greater part of the back- the secondary and tertiary structures mist. One of these introduces to the ground reading for advanced students of transfer RNAs and polysaccharides, student the concept of the chemostat of biochemistry.

There are 21 contributions by 25 different authors, most of them well known in the field of work they present. Of course, certain topics are inevitably sacrificed, and the emphasis is on structural aspects of biochemistry rather than on the traditional metaished interest to the main stream bio- London, June 1974.) £7.00. chemist, such as B. A. Newton's article on "Protozoa as tools for the biochemist", compensate by conveying the systems which are in some respects less tion, hormonal control, immunoglobustyle of a well written textbook.

#### **Biochemical** grand tour

Barry Robson

that unmistakable, if undefinable, Topics for Further Study. Edited by A. are not simply nature's beneficial gift molecular biology flavour. Certain T. Bull, J. R. Lagnado, J. O. Thomas topics which may seem to be of dimin- and K. F. Tipton. Pp. 700. (Longmans:

enthusiasm of the contributer, and well understood. Two excellent articles make surprisingly compulsive reading. together present a particularly compre-Although some chapters sacrifice a hensive review of enzyme kinetics. The certain degree of readability to infor- first of these introduces fast reaction have tried to select topics that are mation content, or vice versa, this is techniques, emphasising their impor- poorly treated in the textbooks, or in no instance well marked, and all tance with the observation that the the contributions are presented in the slowest step in an enzyme reaction typically has a half-life of seven milli- that many teachers will inevitably feel The book falls naturally into two seconds or less. The second presents that neglect of their pet subject has halves. The first deals with the bio- material which will be more familiar not been justified by at least one of genesis, structure, and function of bio- to the average student, but even here polymers. Proteins, nucleic acids, poly- the Michaelis-Menten equation and bably finish the epic journey from saccharides, viruses, and bacterial and Lineweaver-Burk plot are infused with pages 1 to 700 with a sense of complant cell walls are discussed. Three new life in such a way as to dispel the pleteness and the feeling that this parcontributions concern the application notion that the study of enzyme kine- ticular grand tour is a considerable to proteins of chemical modification, tics is "... an esoteric pursuit which tour de force.

The second half of the book is as a device for quantitative investigation of the growth kinetics of cell populations, and skillfully avoids incensing him against yet another "esoteric pursuit". An article which discusses the biochemistry of microbial pathogenicity is dropped right in the middle of these contributions, remindbolic approach. In fact, the book has Companion to Biochemistry: Selected ing the reader that micro-organisms of a research tool.

> The book concludes with a heterogenous mixture of exceptionally good reviews on lysozomes and peroxisomes, mitochondrial oxidative phosphorylalins and the contractile apparatus of muscle.

> The preface states that the editors poorly understood by the average final year undergraduate. Despite the fact these conditions, the student will pro-

#### Living with cosmic rays

Space Radiation Biology and Related Topics. By Tobias and Todd. Pp. xvi+ 648. (Academic, subsidiary of Harcourt Brace Jovanovich: New York and London, May 1974.) £15.85.

It is perhaps surprising to see the publication of this volume now when support for space research is declining. It is, however, soon apparent that the manuscripts for all but the final chapter were conceived and written in the late 1960s when the achievements of the US space programme were at a climax and the future for space research looked a good deal brighter than it does today.

The 12 chapters of the book have been written by 16 authors, including the editors, who are expert in a wide range of subjects relating to space radiation biology After a historical survey, two chapters are concerned with the physics of radiations in space. In the first of these the composition and intensity of the radiation fields encountered around the Earth and in the Solar System are described in detail and the radiation doses received by astronauts taking part in the US missions up to Apollo 11, the first lunar landing, are tabulated. In the second, consideration is given to techniques for the simulation of the heavy-ion component of space radiation fields using accelerators.

From chapter 4 onwards the emphasis is on biology, commencing with a review of cellular radiation biology with special reference to heavy-ion radiations. This is followed by a review of the various theories of molecular and biological evolution, again with special reference to the possible role of solar and galactic radiation. One chapter is concerned with the effects of magnetic field on biological systems and another with the results of experiments carried out in satellites where weightlessness can modify the effects of radiation. The

three following chapters deal with mammalian radiobiology in general, the influence on the biological response to radiation of circadian rhythm and the actue and late effects of radiation observed in man.

Chapter 11 is a discussion of the mathematical models used to described recovery from radiation induced injury and the effects of radiation on ageing The final chapter entitled 'Current Topics ni Space Radiation Biology briefly refers to some of the more important developments that have taken place up to 1972.

The volume is very readable, well referenced and adequately indexed and will no doubt be read with interest by both biologists and physicists working in areas related to space radiation biology. It is, then, a great pity that the reader is so often reminded by, for example, the absence of any mention of the chemistry of interstellar clouds in the chapter on evolution, that the book was written several years ago.

K. F. Baverstock

# North American fish fauna

Freshwater Fishes of Canada. By W. B. Scott and E. J. Crossman. Pp. xi+966. (Fisheries Research Board of Canada Bulletin No. 184.) (Information Canada, Ottawa, 1973.) \$9.75.

THE Fisheries Research Board of Canada has a most creditable publishing record on both fish and fisheries. By commissioning a series of excellent publications on the fishes of the major geographical regions of Canada, published in its Bulletin series, it has made the Canadian fish fauna the best documented in the world. In this, the latest of the series, the authors W. B. Scott and E. J. Crossman faced a formidable task in critically surveying the fishes of northern North America, a fauna which in part is poorly studied biologically and is beset with taxonomic problems, while the remainder (mostly the economically valuable species) has been studied in depth. The authors' achievement in bringing together the information in a large literature (over 1,400 references are cited) is alone a major contribution, and their original work welds the whole together to form one of the best books on a major fish fauna to be published this century.

The plan of the book is essentially a systematic account which commences with a key to the families of freshwater fishes in Canada. This relies on simple characters and is amply illustrated; it is clearly a key designed for use by others than systematic ichthyologists—and is all the better for that. Each family is preceded by a key to the species included in the fauna, and though these are necessarily more technical in nature they are still practical keys. The practical nature of the

means of identification is well illustrated by a page of line drawings devoted to the parr of the species of Oncorhynchus, Salmo, and Salvelinus—confusing the young stages of salmons, trouts, and charr.

Each account is illustrated by a drawing of the fish and has text under headings such as description, colour, systematic notes, distribution, biology, relation to man, nomenclature, etymology and common names. Of these headings, biology is frequently the most detailed, especially in the case of economically important species, and provides data on spawning, growth, age at sexual maturity, habitat, food competitors and predators, and parasites.

The more general parts of the book are confined to a brief note on Canada and its fish fauna, which is essentially a listing of the 181 species by geographical area and drainage basin. This seems to be a rather terse treatment of the zoogeography of Canada's fishes strangely so in contrast with the detailed zoogeographic discussions in McPhail and Lindsey's Freshwater Fishes of north-western Canada and Alaska (1970).

Such criticisms as one needs to make of Scott and Crossman's masterly treatment of the Canadian fish fauna are in extra-limital areas. For example, although most books on European fishes report the occurrence of Ictalurus nebulosus, as introduced to Europe, the only specimens critically examined have proved to be I. melas. Surely too, it is time to forget about the death of Henry I of England in 1135 which is said (page 74) to have been caused by eating lampreys (Petromyzon marinus) -although the lampern (Lampetra fluviatilis) was more likely to have been involved Alwyne Wheeler

# Nice balance for ecologists

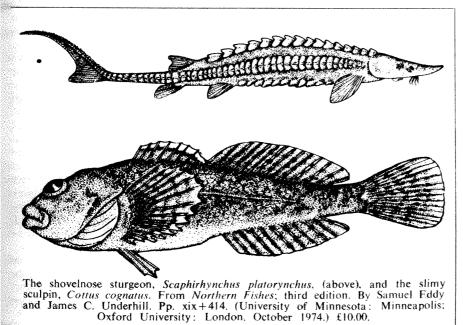
Ecology, with Special Reference to Animals and Man. By Charles Kendeigh. Pp. vi+474. (Prentice-Hall: Englewood Cliffs, New Jersey, 1974.) \$16.95

One of the most frequent criticisms levelled at the examination scripts of modern ecology students is that their knowledge of ecological principles is not accompanied by evidence of experience of living organisms in the field. Ecological field courses were once exercises in field identification; now the recognition of plants and animals has become subservient to studies of ecosystem organisation, trophic structures, population dynamics, pattern analyses, and so on. The pendulum seems to have swung to the other extreme.

Charles Kendeigh's book is unusual in that it attempts to maintain a balance between theoretical principles and detailed accounts of field studies. He achieves this by the insertion of a section which considers certain habitats in detail, including both aquatic and terrestrial examples. By placing this section early in the book, immediately following an introductory section, he demonstrates his conviction that the understanding of ecological principles can only be achieved once the student has become familiar with the diversity of organisms and the complexity of the environment which is to be found in almost any habitat subjected to critical observation. I find this approach both stimulating and refreshing.

There follow three theoretical sections, dealing with ecosystem ecology (nutrient cycling, energy flow, food webs), population ecology and evolutionary ecology. Attempts to simplify language and concept in these sections have occasionally led to misleading expressions, such as the 'energy cycle' and the 'impervious' nature of carbon dioxide and water vapour to long wavelength radiation. Coverage is fairly thorough, however, especially in the section on energy flow, and the relevance of these concepts to the human manipulation and exploitation nature is stressed. The concept of succession is mentioned on several occasions, but a detailed theoretical consideration of this important aspect of ecosystem ecology is lacking.

The coverage of biomes and geographical ecology lacks much of the flow of previous sections. It comprises targely a series of lists of birds and mammals for North American examples of the major biomes. One pleasing feature of these chapters is the inclusion of a few paragraphs dealing with the palaeoecology of the American



biomes, particularly during the Pleisto-

The final chapter, on marine ecology, is rather sketchy and superficial. It gives the impression of being an afterthought.

In my opinion there are three major weaknesses in this book when it is considered as a possible undergraduate text. The first is its lack of information concerning plants. This is particularly noticeable and unfortunate in the chapters on habitat, where it would have completed an otherwise detailed and interesting series of accounts. Although this omission is confessed frankly in the sub-title, it nevertheless reduces the usefulness of the book as an ecological teaching tool. The second weakness from the point of view of British ecologists is its almost exclusive use of North American examples. This must limit severely its value and its sales potential outside the United States and Canada. The third way in which the book could have been improved is by the inclusion of more physiological information about the animals considered. I cannot agree with the author's statement in the Preface that such information is only of value at advanced levels of training.

Despite these reservations I found the style, content and production of this book attractive and interesting.

Peter D. Moore

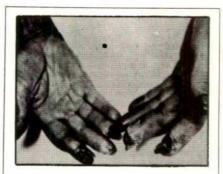
# What is biological control?

Biological Control of Plant Pathogens. By K. F. Baker, and R. J. Cook. Pp. xiv+433. (Freeman: San Francisco, 1974.) \$12.50.

THE authors of this book admit that writing it was a "mind-stretching" exercise; many readers may think that the effort has somewhat distended their subject. The dilemma faced by the authors was how far to extend beyond the difficulty of defining 'biological control' into the wider problems of managing microbial ecology to lessen plant diseases. Early usage of the term fitted well into S. D. Garrett's statement (in Pathogenic root-infecting fungi, Cambridge University Press that biological control is "mediated by one or more organisms (excepting man himself) outside the host-parasite relationship". On page 43 of this book the authors arrive at their much broader and wordier concept: "Biological control is the reduction of inoculum density or diseaseproducing activities of a pathogen or parasite in its active or dormant state by one or more organisms, accomplished naturally or through manipulation of the environment, host or

antagonist, or by mass introduction of one or more antagonists."

This concept is so broad that it embraces all of microbial ecology and much of agriculture, except the use of chemicals aimed solely against pathogens. It is of course commendable that plant pathologists should consider all the factors affecting the biology of plant pathogens but, despite the inevitable demarcation disputes, many would share my preference to restrict the term 'biological control' to the manipulation of 'third organisms' such as hyperparasites, antagonists and competitors. A more comprehensive term would be needed to comprise the important natural environmental artificial factors which



The hands of a workman who had been operating a pneumatic drill for 26 years. The prolonged vibration caused stiffness swelling and, finally, dry gangrene in the fingers. From *The Vibration Syndrome*. Edited by W. Taylor. Pp. xii+226. (Academic Press: London, August 1974.) £6.00; \$15.50.

included as biological in this book; for example, tillage, choice of planting date, and organic and inorganic additives (including fertilisers). In Chapter 9 the authors come perilously close to accepting chemotherapeutants as another factor, thus removing the chief objection to adopting the well established term 'integrated control'.

The first two chapters contain a somewhat unhappy blend of unnecessarily elementary introduction, good sense, and an emotional attitude to unbalanced 'environmentalist' arguments. Both authors have, however, enviable reputations as root pathologists and most of the book comprises a skilled and up to date compilation of knowledge concerning root pathogens. It is comforting to see one chapter devoted to pathogens of aerial parts; but that occupies a mere 20 of the 350 pages of text and so can be no more than an apology to satisfy the completeness that the title suggests. In their preface the authors disclaim comprehensiveness and admit that they have often put a personal interpretation on results which is different from that of the authors they quote. That this is a risky procedure is reinforced

by several authors who claim to have been misrepresented, which breeds doubts about inaccuracies elsewhere.

Inevitably, much of the evidence from the literature is anecdotal and biased, because successes are more often reported than failures (which may be more frequent). With a few notable exceptions it is therefore very difficult to obtain balanced estimates of the value to agriculture of modifications to microbial ecology or control by 'third organisms'. Experience with the decline phenomenon in take-all disease of cereals (Gaumannomyces graminis) suggests that the variability between cropping histories, soils and fields is so great that it will be long before we can confidently predict the effects of antagonists or of developments in biological buffering. These are not, however, reasons for criticising a book that collects together much scattered information, presents it systematically for the first time, and contains more than the usual amount of constructive thinking.

Plainly, the authors did not want this to be an ordinary book; it is not. It will periodically delight, interest and infuriate its readers; but these seem to be characteristics of the books that prove most formative to their subjects and I think time will show this book to be important.

J. M. Hirst

#### Of mites and models

The Functional Response to Prey Density in an Acarine System. (Simulation Monographs.) By H. G. Fransz. Pp. viii+143. (Centre for Agricultural Publishing and Documentation: Wageningen, 1974.) Dfl.20.

DURING the last few years ecological theory seems to have outpaced the collection of ecological data. Professor Maynard Smith's book Models in Ecology (Cambridge University Press, 1974) showed amply that models can be formulated and built, but that their testing on real data and validation by prediction in the real world is still not possible because of a shortage of data. It was with these thoughts in mind that I welcomed Fransz's book which describes systems whereby the mite Typhlodromus occidentalis preys on various stages of another mite Tetranychus urticae. The book combines the observational approach to this predatorprev system with model building and validation by comparison with experimental results.

The first two chapters provide an introduction to the experimental system and the variables that the author measured (prey density, hunger, locomotion velocity and so on), and the third chapter describes the results of

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experiments that measured these variables. Multiple regression analysis, apparently without significance tests, is frequently used to relate the different variables. The fourth chapter is concerned with building models, and the final chapter compares the output of models with the experimental data, demonstrating that stochastic models generally give a better fit to data than deterministic models.

The book does achieve the objective of building and testing models, but its style closely resembles the thesis that it is. The use of Fortran-type variables throughout the text and in tables and figures makes the book difficult to read, necessitating continual reference to Appendix IV where one needs to look up the root variable name as well as prefixes and suffixes. The information in the book is diffuse and space is wasted with unnecessary simple computer programmes and procedures. The essential points of model building and of testing on experimental data could have been written much more concisely, and probably with more effect, as an article in a journal.

M. B. Usher

#### **Drugs and toxins**

The Chemistry, Metabolism of Drugs and Toxins: An introduction to Xenobiochemistry. By Michael and Maxine Briggs. Pp. xii+386. (Heinemann Medical: London, June 1974.) £5.00.

This pot-pourri of data from chemistry, biochemistry, biochemical pharmacology, pharmacology and toxicology is put together in four chapters entitled, respectively, Metabolism of foreign compounds, Biochemistry of drugs, Natural antimetabolites, and Venoms. It is given the sub-title "Xenobiochemistry" which is not new (see Nature, 187, 94; 1960). The first chapter contains a somewhat superficial account of drug metabolism and the second tries to cover the types, absorption, transport, elimination, mode of action and side effects of drugs, but has 50 pages of tables with several half-empty pages. The third chapter covers microbial toxins, antibodies and antimetabolites whereas the fourth is on the composition and action of all kinds of animal and plant venoms. The book has attempted to concentrate too much into too little and this results in some wrong impressions, as in the case of thalidomide (p. 192) which is known to undergo spontaneous hydrolysis in vivo and in vitro rather than the enzymic metabolism implied by the book. The chapters on antimetabolites and venoms are, however, useful introductions to the study of these toxins.

R. T. Williams

(Continued from page 128)

eluted the agglutinin with D-galactose (ref. 22 and details to be published). Based on specific agglutination titre a 75-fold purification with 50% recovery was achieved. Since the product was pure by electrophoretic criteria (Fig. 2) this result indicates that the agglutinin constitutes approximately 1% of the total extractable protein in cohesive P. pallidum cells. The subunit molecular weight of the P. pallidum protein is estimated to be about 25,000 (Fig. 2) as compared with 26,000 for discoidin11; its isoelectric point is 7.0 (details to be published) whereas the value is 6.1 for discoidin11. The two agglutinins also differed in their interaction with a wide range of sugars (Table 1). For discoidin, the most potent inhibitors were N-acetyl-D-galactosamine and 3-O-methyl D-glucose. In contrast, the P. pallidum agglutinin required 15 times as much of the former and 60 times as much of the latter for comparable inhibition but was eight times more sensitive to lactose and four times more sensitive to D-galactose.

Since the protein agglutinated erythrocytes we determined if it also agglutinated *P. pallidum* cells. To test this we added the pure protein to cohesive *P. pallidum* cells which had been

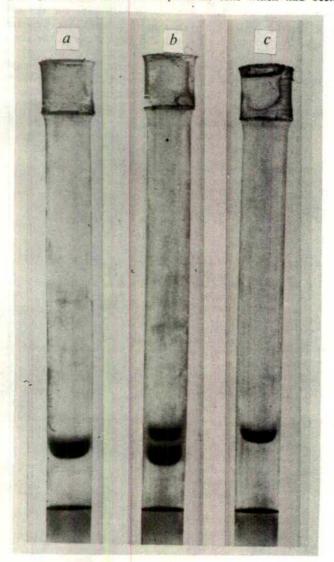


Fig. 2 Polyacrylamide gel electrophoresis of purified agglutinins from *P. pallidum* and *D. discoideum*. Approximately 25 μg of each protein was applied to a discontinuous sodium dodecyl sulphate (SDS)-polyacrylamide system<sup>23</sup> with a 3% acrylamide stacking gel and an 8.0% separating gel as described previously<sup>11</sup>. To assure complete solubilisation, samples were heated at 100° C for 5 min immediately before application to gels. Cytochrome *c* (5 μg) was added routinely to samples as a reference protein. The discoidin preparation and molecular weight calibration were as described previously<sup>11</sup>. *a*, *P. pallidum*; *b*, mixture; *c*, *D. discoideum*.

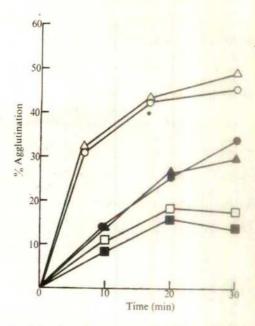


Fig. 3 Agglutination of heat-treated (60° C) *P. pallidum* cells by purified agglutinin. Cells were collected from 90-h plates, washed and suspended, 10° ml<sup>-1</sup>, in 16.7 mM Na<sub>2</sub>HPO<sub>4</sub>-KH<sub>2</sub>PO<sub>4</sub> (*p*H 6.0) buffer, and heated at 60° C for 10 min. Agglutinin was purified and then extensively dialysed against PBS. Since there was partial loss of activity, the concentration of active agglutinin was estimated on the basis of agglutination activity. Agglutination assays were done in plastic trays with 16 mm wells (Linbro cells ml<sup>-1</sup> plus one of the following sets of consisting of 2×10° cells ml<sup>-1</sup> plus one of the following sets of constituents: (Δ) PBS; (Δ) purified agglutinin (0.5 μg ml<sup>-1</sup>)+D-galactose (0.2 M); (□) purified agglutinin (0.5 μg ml<sup>-1</sup>)+D-glucose (0.2 M); (□) 0.2 M galactose; (♠) 0.2 M D-glucose. A separate well was used for each time point. The tray was gyrated at 115 r.p.m. on a New Brunswick G-24 shaker (23° C). At 10 min intervals the contents of wells were carefully removed, diluted in 20 ml of saline and single cells were counted with a Coulter counter (100 μm aperture: 1/aperture current = 1/4; 1/amplitude=2; threshold=20-80. The percentage agglutination was expressed as the percentage of single cells that had disappeared relative to time 0 (ref. 24).

heated at 60° C for 10 min to reduce their endogenous agglutination (Fig. 3). Agglutination was assayed by measuring the disappearance of single cells in a gyrated suspension 24.2 The purified protein (0.5 µg ml<sup>-1</sup>) agglutinated the *P. pallidur* cells; and this agglutination was blocked by 0.2 M p-galactose but not by 0.2 M p-glucose (Fig. 3).

We next looked for agglutinin on the surface of cohesive cells. When erythrocytes and cohesive P. pallidum cells were mixed and shaken on a slide, large mixed clumps formed within a minute, consisting of slime mould cells in contact with one another and with erythrocytes. p-Galactose (0.15 M blocked formation of these clumps whereas p-glucose did not To test the possibility that erythrocytes were being agglutinate because of secretion or release of protein from the slime mould cells, we maintained cells in medium for 10 min, then sputhem out, and tested the supernatant for erythrocyte agglutination activity. Since no agglutination was observed, this suggested that the agglutinin is not secreted and is therefor present on the surface of the slime mould cells.

In view of this finding we investigated whether the saccharide that interact with this protein blocked the endogenous cohesive ness of the slime mould cells. In a previous experiment (Fig. 3 we found that cells heated to 60° C for 10 min retained som cohesiveness. With a milder heat treatment (51° C for 10 min the cells were significantly more cohesive. (It should be note that purified factor retained about 50% of its erythrocyt agglutination activity when heated under these conditions. Determining agglutination by measuring the disappearance of single cells, we examined the effects of several sugars on this residual cohesiveness. D-Galactose and lactose were effective

% Inhibition of agglutination

inhibitors down to 6 mM whereas p-glucose and p-mannose were effective only at 500 mM (Fig. 4a). When living, untreated P. pallidum cells were used, differential sugar inhibition of cohesiveness was again observed (Fig. 4b and c); but compared with the results with heat-treated cells, the concentrations of sugars required for differential inhibition were about 15 times higher. The difference between heat treated and living cells may be due to (a) more functional agglutinin in living cells; (b) higher binding affinity between agglutinin and its receptor in living cells; or (c) secondary adjunct binding sites in living cells.

Our findings support a role for the carbohydrate-binding protein in intercellular adhesion in P. pallidum, although its participation in other differentiative processes should be

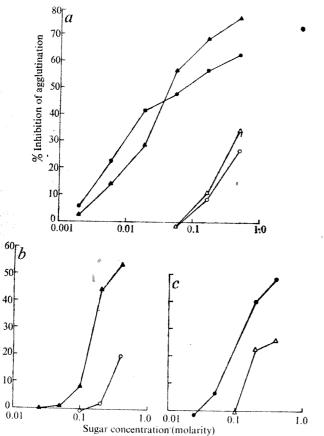


Fig. 4 Effect of sugars on agglutination of heat-treated (51° C) (a) or normal (b, and c) P. pallidum cells. a, Cells from 90 h plates were suspended at 10<sup>7</sup> ml<sup>-1</sup> in 16.7 mM Na<sub>2</sub>HPO<sub>4</sub>-KH<sub>2</sub>PO<sub>4</sub> pH 6.0, buffer and heated at 51° C for 10 min. The cells were then washed and dispersed in the same buffer. Each well of the Linbro tray contained 2×10<sup>6</sup> cells ml<sup>-1</sup> plus PBS buffer containing the indicated concentration of sugar (0.5 ml total volume) taining the indicated concentration of sugar (0.5 ml total volume). There were three replicate wells for each treatment. The plate was gyrated at 115 r.p.m. for 60 min, by which time agglutination had reached a plateau. The contents of each well were diluted and counted as in Fig. 3 to determine the percentage reduction in agglutination relative to controls containing no sugar. The selective inhibition of agglutination seen quantitatively in the figure was verified qualitatively by visual inspection (b and c). Cells were collected from 93-96 h plates. The cells were suspended and dispersed into EDTA-phosphate buffer (Fig. 1 legend). Each well of the Linbro tray contained  $3 \times 10^6$  cells ml<sup>-1</sup> plus EDTA-phosphate buffer or sugar in EDTA-phosphate buffer at different concentration (0.5 ml total volume). Galactose ( $\triangle$ ) and mannose  $(\bigcirc)$  were compared in one experiment (b) and lactose (lacktriangle) and glucose  $(\triangle)$  in the other (c). There were three 115 r.p.m. for 60 min, a period sufficient to attain equilibrium. The contents of each well were diluted into 20 ml of EDTA-phosphate buffer and single cells were counted (100 µm aperture; 1/aperture current=0.354, 1 A=1, thresholds=10-60). As above, the percentage reduction in agglutination was determined for each sugar. The selection counter of the sugar that for each sugar. The selective sugar effects were verified visually.

considered. Our working hypothesis is that this protein is a cell-ligand26 that binds slime mould amoebae together by attaching to carbohydrate-containing receptors on adjacent cells. The basis of the erythrocyte agglutination assay would derive from a structural resemblance between the erythrocyte surface oligosaccharides and the native slime mould receptor. Thus, under this model, there would be two principal components in the adhesion system: a multivalent carbohydratebinding protein which is a peripheral membrane protein<sup>27</sup> and a carbohydrate receptor possibly associated with an integral membrane component<sup>27</sup>. Carbohydrate-containing macromolecules have been implicated in cell adhesion in several other systems, including microbe-host cell interactions 18-30, mating reactions of bacteria<sup>31</sup>, yeast<sup>32</sup> and Chlamydomonas<sup>33</sup> and cell-cell interactions in tissue formation3,34-36.

Finally, we suggest that the difference in the carbohydratebinding specificities of the agglutinins from D. discoideum and P. pallidum may underlie the selective intercellular affinities exhibited by cells of these species 37-39. Further work is required to test our model for slime moulds and to determine its relevance in the analysis of specific cellular interactions in higher systems.

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#### Cyclic expression of a growth conditioning factor (MGF) on the cell surface

THE cell surface has been implicated as a regulation site for cell division in various systems. Enzymatic alteration of the cell surface can initiate DNA synthesis in contact inhibited cells1-3 and changes in membrane components have been correlated with the expression of the transformed phenotype4-10 and with the entry into the S phase of normal cells11. We describe here a system in which the induction into DNA synthesis of a nondividing cell population is regulated by a factor exteriorised only during the S and mitosis periods at the surface of the producer cells.

Peritoneal macrophages can be maintained in vitro but do not proliferate; they can, however, be induced to synthesise DNA and divide in medium conditioned by cells of the same species12-14. In our system medium conditioned by L cells in logarithmic growth was used and tested on macrophages collected from the peritoneal cavities of starch-inoculated C57BL/6J mice as described previously13. The assays were performed in Lab-Tek four chamber slides seeded with 3.0  $\times$  10 $^{\rm 5}$  nucleated peritoneal cells per chamber. Thirty minutes after seeding, the cultures were washed twice with phosphate buffered saline (PBS) to remove unattached cells, and refed. After 2-3 d the desired concentration of samples was diluted in TES-HEPES-buffered Eagle's medium. pH 7.6 (ref. 15), with 10% foetal calf serum (FCS) and added to macrophages in the presence of 0.2 μCi ml<sup>-1</sup> <sup>3</sup>H-thymidine (dT) (specific activity, 52 Ci mmol<sup>-1</sup>). After 5 d the cells were fixed with Carnoy's solution and prepared for autoradiography.

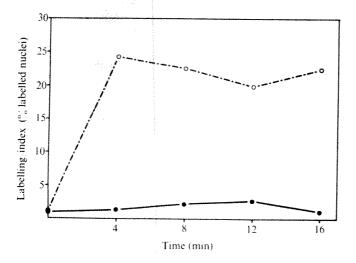


Fig. 1 Release of MGF with increasing time of trypsin treatment. Incubation of 2 ml of 0.1% trypsin (○---○) or medium alone (●--●) with duplicate milk dilution bottles of L cells previously washed twice with PBS. Reaction stopped with 10% FCS. All samples assayed in duplicate at a trypsin digest concentration of 40%. Assay was for 5 d with a continuous label of 0.2 μCi ml<sup>-1</sup> dT.

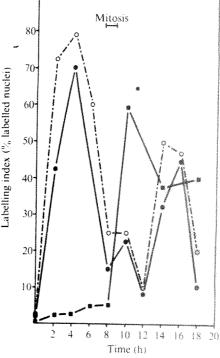


Fig. 2 Spontaneous and tryptic release of MGF from synchronised L cells. L cells seeded 24 h previously, 1 × 10° cells per 60 mm Petri dish were synchronised with 10 ° M dT as described. in the text. At different times after release, L cell coverslips were pulsed for 20 min with 2.0 µCi ml<sup>-1</sup> dT (specific activity 52 Ci mmol<sup>-1</sup>). Coverslips were fixed in Carnoy's solution for autoradiography. Cells were read for labelled nuclei ( times indicated duplicate L cell cultures were trypsinised with 0.1% trypsin for 5 min and the reaction was stopped with 10% FCS. Samples of medium incubated with the cells from the time and conditioned medium ( $\blacksquare - \blacksquare$ ) were assayed on macrophages at a concentration of 40% for 5 d with 0.2  $\mu$ Ci ml<sup>-1</sup>dT. Autoradiography was performed as described previously.

The presence of macrophage growth factor (MGF) not only in the conditioned medium, but also in cell lysates was demonstrated as follows. Suspensions of 4 × 10° L cells per ml were washed repeatedly and sonicated for 3 min at 30-s intervals. Dilutions of whole sonicates were assayed on macrophages for stimulatory activity. An increase in the concentration of crude cell lysates increased the number of labelled macrophages. To determine whether stimulation was specific, two mouse cell lines which do not produce active conditioned medium were sonicated and tested for activity: neither showed stimulatory activity in their lysates (data not shown).

To determine the cellular localisation of the MGF, L cells were treated with 0.1 % trypsin (Flow Laboratories) 24 h\*after plating at 37° C for periods of 0-16 min; trypsinisation was terminated with 10% FCS or soybean trypsin inhibitor. Samples were filtered through 0.22 nm filters to eliminate contaminating L cells. As Fig. 1 shows, maximal activity was released at 4 min; 0.05% purified trypsin (Worthington) released comparable amounts of activity (data not shown). No detectable MGF was released when cells were incubated with medium alone during the experimental period.

Since changes occur in the components of the cell surface during the cell cycle we investigated whether MGF was released by trypsin only during certain phases of the cycle. L cells were synchronised by a double dT block (10-3 M dT for 16 h, 6 h release, followed by another 16 h block). At 2 h after release, 70% of the cells were in the S phase as shown by autoradiography after a 20-s pulse with dT (Fig. 2), Mitosis (30%) followed 8 h after release; a second round of DNA synthesis followed 12 h after the first.

At different times in the cell cycle samples of medium were taken to test for spontaneously released MGF, and L cells were

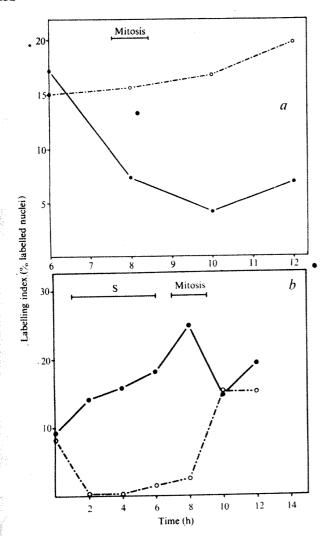


Fig. 3 a, Trypsin release of MGF in cells blocked during mitosis. L cells synchronised with a double dT block were released (time 0) and 5 h later blocked with 0.03 µg ml<sup>-1</sup> vinblastine sulphate (Velban, Lilly) (\(\circ\) - - \(\circ\)) or refed with medium alone (\(\bullet\)-\(\bullet\)). At the times shown, 5 min duplicate trypsin digests were taken. Samples were assayed at a concentration of 40° under standard conditions. b, MGF in lysates from synchronised L cells. L cells synchronised by a double dT block as described in the text were scraped from two 60-mm Petri dishes into 2 ml of TES-HEPES-buffered Eagle's medium (pH 7.6) with 10% FCS and sonicated for 30-s intervals totalling 3 min. ——, Points were taken every 2 h from time of release from dT. At the same times, sister cultures were trypsinised for 5 min (this did not remove cells from the glass). These cells were then treated similarly for sonication. Ο --- Ο, All sonicates were assayed at a concentration of 10% for 5 d in the continuous presence of 0.2 μCi ml<sup>-1</sup> dT. Slides were prepared for autoradiography as described previously.

trypsinised for 5 min with 0.1% trypsin at 37° C. Under these conditions, there was no alteration in cell morphology and viability remained at 95%. Figure 2 shows that MGF activity could be trypsin-released from cells only during the S phase, with almost no activity recoverable when DNA synthesis stopped; activity reappeared again at the next S phase. The spontaneously released MGF appeared in the medium as the activity disappeared from the surface.

It was not clear in these experiments whether MGF was released before or after mitosis. To resolve this, L cells were synchronised with a double dT block, then released, and 5 h later, in the middle of S phase, arrested with 0.03 g ml<sup>-1</sup> of vinblastine sulphate. In the control cells, as expected, no MGF was released by trypsin during G1; in the cells blocked in mitosis instead, MGF could be released as late as 12 h after removal of the dT block (Fig. 3a). It seemed therefore that MGF remained associated with the cell surface in cells blocked at mitosis.

The above results indicated cell cycle dependency for the presence of MGF in the trypsin-releasable material both at the cell surface and in the medium. To understand better the dynamics of the distribution of MGF during the cycle, localisation in the whole cell lysate was compared with that remaining in the cells after trypsin treatment. Parallel cultures were synchronised as before. MGF was assayed in the whole lysate from cultures previously treated with trypsin or untreated. All points were taken from duplicate assays of lysates from two 60-mm Petri dishes each containing  $3 \times 10^6$  to  $4 \times 10^6$  L cells. Figure 3b shows that MGF activity was present in cell lysates at all times in the cell cycle. Trypsinisation of the cells before sonication eliminated activity in lysates only during S and mitosis, confirming previous results with trypsin digests.

On the basis of the results of this study, the following interpretation can be made: (1) MGF is present and presumably synthesised at all times in the cell cycle; the buildup of activity seen before transfer to the surface and release supports this. (2) MGF is transferred to the surface during S phase, where it remains until after metaphase when it is released into the medium.

Previous work in our laboratory has shown that MGF present in L cell-conditioned medium was stable to various enzymes and treatments, but was destroyed by periodate and by subjection to  $100^{\circ}$  C for 10 min (ref. 12). These studies indicate that the active moiety contains carbohydrate. This agrees with evidence that trypsin releases glycopeptides from the surface of plasma membranes (for a review see ref. 22). The mode of synthesis and transfer of this factor as described here is in accord with the model of synthesis of glycoproteins proposed by Schachter and Roden<sup>16</sup>. These authors reported that glycoproteins are synthesised intracellularly on polyribosomes and as glycoproteins transported through the Golgi apparatus to the cell periphery16. Several growth regulatory factors have been isolated and identified as glycoproteins, attesting to the significance of this class of molecules in the control of cell proliferation17-20.

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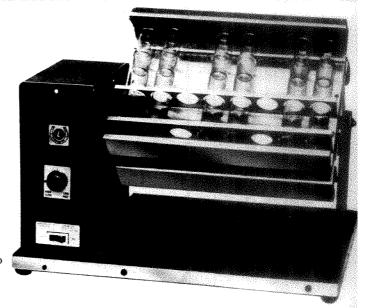
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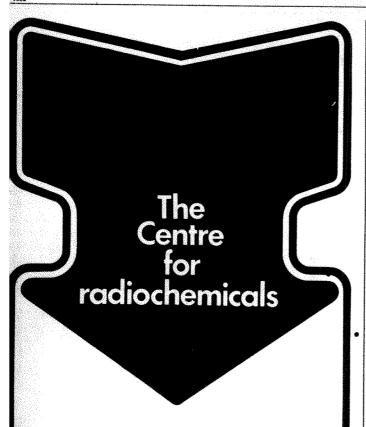
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#### An electrophysiological study of the hippocampus of the 'reeler' mutant mouse

THE laminar organisation of neurones in the brain of the homozygous 'reeler' mutant mouse1 is grossly disrupted, especially in the cerebellum, hippocampus and neocortex2-6. Although a number of histological studies on synaptic organisation in the cerebellum of various mutants have been reported7-9, the effect of such disorganisation on interneuronal connections has not been investigated electrophysiologically, either in the cerebellum or elsewhere.

Here we report evidence which suggests that there is a high safety factor in the developmental processes responsible for ensuring orderly synaptic organisation. Despite the cellular disarray in the hippocampus of the homozygous reeler, the resultant synaptic organisation as revealed by electrophysiological techniques is essentially normal.

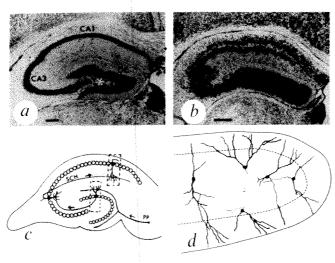


Fig. 1 Anatomy of normal and reeler hippocampus. Transverse sections through the hippocampal formation of 18-d-old normal (a) and 'reeler' (b) littermates. Cresyl fast violet stain. Asterisk indicates the hilus of the dentate area. CA1 and CA3, pyramidal cell fields CA1 and CA3 of Lorente de Nó (1934); GC, granule cells. Bars in a and b denote 200 µm. c, Diagram of the main excitatory pathways in the hippocampal formation. Arrows give the direction of impulse traffic. Dashed rectangles represent approximate regions of the hippocampal formation from which the data of Figs 2 and 3 were obtained. PP: perforant path; SCH: Schaffer collaterals, d, Composite camera lucida drawing of granule cells in the dentate area of 'reeler' mice, impregnated by the rapid Golgi method. Dashed outline indicates the boundary of the region occupied by granule cell bodies. Bar denotes 100 µm.

The extent of the defective alignment of cells in the hippocampus is shown in Fig. 1. In the normal animal both the pyramidal cells of regions CA3 and CA1 and the granule cells of the dentate area are arranged in tightly packed layers. In the reeler, the rigid layering of cells seen in the normal animal is absent; pyramidal cells are loosely packed, with occasional discontinuities and in region CA1 form two distinct layers, while granule cells are scattered widely throughout the hilar region of the dentate area.

The synaptic organisation of the normal hippocampus has been studied both histologically<sup>10</sup> and electrophysiologically<sup>11, 12</sup>. The axons of the perforant path (PP in Fig. 1c), the main input

to the hippocampal formation, form en passant synaptic contacts with the dendrites of granule cells. Pyramidal cells in region CA3 of the hippocampus, to which axons of the granule cells project, extend myelinated Schaffer collaterals (SCH in Fig. 1c) to the apical dendrites of pyramidal cells in region CAL

We have investigated the synaptic organisation of the perforant path and Schaffer collateral systems in reeler using the technique of field potential analysis. Wild-type or heterozygous littermates were used as controls.

When the perforant path of the normal animal is electrically stimulated, the synchronous depolarisation of the granule cel dendrites results in an inward flow of membrane current in the synaptic region; this inward current is passively extruded from the unactivated regions of the cell population, principally from the cell body layer. With an extracellular electrode, the distribution of membrane current is reflected as a negative potential in the region of the active current sink, reversing to a positive potential as the electrode approaches the cell body layer. A curve conventionally referred to as the 'depth profile of the response can be obtained by plotting the amplitude of the

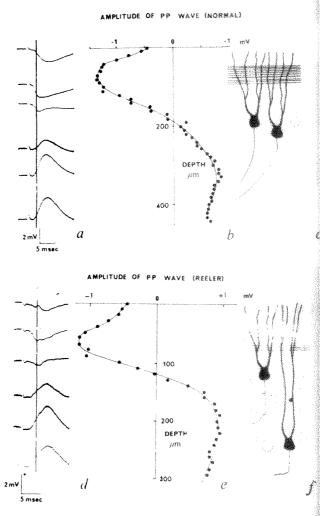
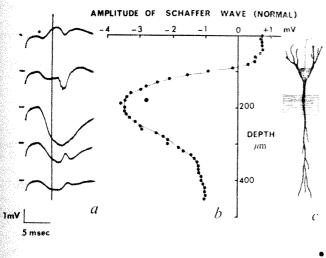


Fig. 2 Depth profiles of the perforant path (PP) wave in normal (a-c) and reeler (d-f) littermates. Samples of the synaptic waveforms are displayed on the left (a, d); the position of the baseline corresponds to the depth at which each potential was obtained. The amplitude of the evoked waveform, at a fixed latency (solid vertical line in a, d), is plotted as a function of depth in b and e. Zero depth was taken arbitrarily as 70  $\mu$ m above the point of maximum negativity, corresponding approximately to the hippocampal fissure. A spot of dye was ionto-phoretically ejected from the recording electrode at the end of each experiment. This gave a reference point enabling the position of the cell bodies to be accurately aligned with the corresponding depth profile (c, f). All animals were aged between 18 and 21 d and were anaesthetised with Equithesin.



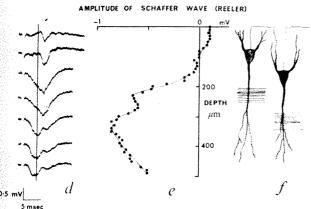


Fig. 3 Depth profiles of Schaffer waves in normal (a-c) and reeler (d-f) littermates. Samples of waveforms elicited by stimulating the Schaffer collaterals are displayed in a and d. The amplitude of the evoked waveform at a fixed latency (solid vertical line) is plotted as a function of depth from the hippocampal surface (b and e). The relative positions of the pyramidal cell population and Schaffer fibres are illustrated in c and f.

evoked potential, at a fixed latency, as a function of depth. In Fig. 2b, a profile of this type, obtained by stimulating the perforant path in a normal mouse, is plotted together with epresentative responses at various depths (Fig. 2a). The elative position of the granule cells is illustrated diagramnatically in Fig. 2c.

The depth profile of the perforant path response in the eeler (Fig. 2e) is essentially similar to that seen in the normal enimal. As in the normal, the evoked potential is negative in the molecular layer, superficial to the region of scattered granule tells, and its polarity reverses as the electrode advances to the ell body region. This result, which was consistently obtained in eelers, shows that perforant path terminals occupy the same osition in the reeler as they do in the normal animal. Two additional lines of evidence indicate that displaced granule ells establish synaptic contact with the perforant path by extending their dendrites into the molecular layer. First, ecords were obtained from single cells in the dentate area; in eelers, cells responding to stimulation of the perforant path ould be found throughout the hilar region, whereas in normal mice such neurones were confined to the granule layer. Secondy, we compared Golgi impregnated material in normal and celer mice. In the normal animal, granule cells were restricted the granule layer; in reeler, granule cells were frequently een in the hilus, with abnormally elongated dendritic arborisaions, sometimes extending into both upper and lower blades of the molecular layer (Fig. 1d).

Pyramidal cells of region CA1 were activated by stimulation of the Schaffer collaterals. In the normal animal, the peak of the

synaptic region occurs approximately  $100~\mu m$  below the pyramidal layer (Fig. 3b, c). In reeler, the pyramidal cell layer is split into two sublayers, separated by  $100~\mu m$  (Figs 1b~and~3f). The depth profiles obtained from reelers contained a double peak (Fig. 3e), showing that Schaffer collaterals form synaptic contacts with the dendritic shafts of pyramidal cells in two distinct layers, separated by the same distance as the cell body layers. This suggests that a different mechanism is at work in maintaining the integrity of the Schaffer projection from that discussed above for the perforant path. Instead of the post-synaptic cells extending their dendrites, the afferent fibres adjust their course to innervate the appropriate dendritic section of the displaced cells, either by means of a diversion of part of the Schaffer bundle to lower than normal depths, or by collateral sprouting.

The development of the hippocampus involves a number of events occurring in sequence. Autoradiographic analysis13 has shown that pyramidal cells are generated mainly between the 12th and 15th day prenatally, while granule cells mitose from the 16th prenatal day until the end of the third postnatal week. From the germinal layer, neurones migrate to their eventual destination and form laminar aggregates before making functional connections between one neurone population and another. In the rat, synaptic connections reach adult levels of efficacy by the end of the third postnatal week<sup>14</sup>. The events which lead to cellular disarray in the reeler brain presumably occur during, or subsequent to cell migration<sup>15</sup>. The site of the primary lesion of mutant gene action is not known; for example the migrating cells may be deprived of a genetic instruction to reach a given site, or they may be devoid of a surface property which facilitates aggregation once they have arrived; cortical cells cultured in vitro from reeler mice do not aggregate to form the laminar arrays characteristic of cells from normal littermates16.17.

Our present findings show that in reeler mice, orderly synaptic connections can be made between neurone populations in the hippocampus in spite of a genetic lesion affecting a prior developmental stage. When a granule cell is displaced in the hilar region of the dentate area, the dendritic tree of the cell is extended to the region traversed by the perforant path fibres. This suggests that the dendrites of displaced cells continue to grow until specified synaptic contacts are made. An alternative kind of adjustment to spatial disorganisation is seen in region CA1, where malpositioned pyramidal cells receive a normal Schaffer input. Analysis of the depth profiles suggests that a segment of the dendritic shaft at a given distance from the cell body has a high affinity for Schaffer fibres, and that when these segments are misaligned, afferent fibres are diverted, or form collaterals, to make synaptic junctions with the appropriate region of the postsynaptic cell. Thus, there is a high safety factor in the overall developmental programme for the hippocampus which ensures that the effects of a previous error are minimised.

We thank Dr R. Victoria Stirling for preparing the Golgi material and M. L. Errington and Maria Utting for technical assistance.

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#### Different spike mechanisms in axon and soma of molluscan neurone

STUDIES with a few invertebrate preparations have suggested different ionic mechanisms for spike generation in the soma and axon of the same neurone. Iwasaki and Satowi found that somatic spikes in the crayfish eyestalk X organ had a mixed Na-Ca dependency, while the extracellular axon spikes disappeared in Na-free or tetrodotoxin (TTX)-containing solutions. Wald<sup>2</sup> recorded attenuated axon spikes in hyperpolarised somata of snail ganglion cells and showed that they varied in amplitude with external sodium concentration but, curiously, were not blocked by TTX. In voltage-clamp experiments with the left pleural giant cell in Aplysia, Kado<sup>3</sup> found that early inward currents in the axon-hillock region were much more sensitive to TTX than early inward currents in the opposite end of the soma, suggesting a greater Na-dependency of the axon spike.

In none of these studies, however, were action potentials recorded with intracellular electrodes in the axon at a large distance from the cell soma. We have been able to record from the axon of the right giant (R2) cell in the visceral ganglion of Aplysia at a distance of 3-4 cm from the soma. Our measurements indicate that the axon length constant is about 1 cm, so the axonal and somatic recording sites are electrically remote. This direct recording technique confirms that the axon spike is mainly sodium dependent, while the somatic spike has both sodium and calcium components4-6.

In all experiments the visceral ganglion together with the right (dorsal view) visceropleural connective was dissected from the animal and placed in a small-volume recording chamber containing normal saline. The preparation was then treated with a 1% solution of pronase (Calbiochem) in normal saline for 15-20 min to facilitate impalement through the epineurium. The microelectrodes used had resistances from 3-8 M $\Omega$ . Standard intracellular recording techniques were used, and all experiments were performed at room temperature.

The R2 axon is visually indistinguishable from the other axons in the connective because of its small diameter (35 µm) and lack of pigmentation7. Thus, we usually identified the axon by stimulating the soma with regular (1 s<sup>-1</sup>) pulses while searching across the right connective with a recording electrode at least 3 cm distal to the soma. Identification of the axon was made when both a 40-60 mV resting potential and 70-90 mV spike with a constant delay after the somatic spike were recorded with the axon electrode. The conduction velocity from soma to axon was typically 1 m s<sup>-1</sup>. In some experiments, stimuli were applied to the right connective with a suction electrode. This method was more convenient than attempting to place a second intracellular stimulating electrode in the axon. The suction electrode also enabled us to depolarise the axon more than was possible with a subtracting circuit for stimulating through the recording

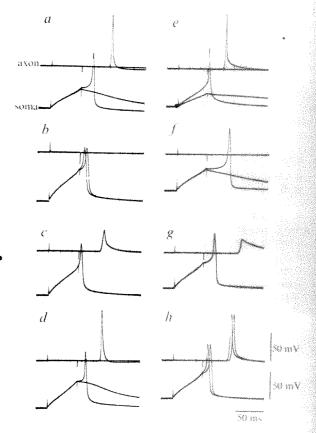


Fig. 1 Responses to somatic stimulation measured in soma and axon at 3 cm from soma. Two sweeps superimposed in most pictures. a, Normal saline; b, Na-free (Tris) solution; c, 2 min after perfusion with normal saline again; d, normal saline, 5 min later; e, normal saline; f, Na-free, Ca 340 mM; g, 2 min after perfusion with normal saline again; h, normal saline, 5 min later. All data from the same cell.

electrode. The R2 cell axon was apparently the only one from which we could record intracellularly, as no smaller spikes of large variations of conduction velocity were seen with antidromic stimulation.

Solution changes were made by perfusing about 40× 11 chamber volume of each solution. The normal saline used has the composition: NaCl 494 mM, KCl 11 mM, CaCls 11 mN MgCl<sub>2</sub> 19 mM, MgSO<sub>4</sub> 30 mM, Tris-HCl (pH 7.7) 10 mM Sodium-free saline had the same composition except that 5 mM neutralised Tris was substituted for sodium. High-calcium saline was made by replacing the NaCl with 340 mM CaCl, an substituting MgSO<sub>4</sub> with MgCl<sub>2</sub> to prevent precipitation of CaSO<sub>4</sub>.

The mixed Na-Ca dependency of the somatic action potential in the R2 cell has been shown previously4-6. The ionic depend ency of the axon spike is compared with that of the somat spike in Fig. 1. The top trace in each part is the axon membrar potential measured at about 3 cm from the soma, and the bottom trace is the somatic membrane potential. Depolarising stimu were applied to the soma through a second intracellula electrode. Figure 1a was obtained in normal saline, and show two superimposed sweeps, one in which the stimulus was su threshold, and one in which it was suprathreshold. The axon spil was 87 mV, and the somatic spike was 93 mV, measured from resting. Figure 1b shows the blockage of the axon spike which was seen after about 2 min in Na-free (Tris) medium, while the somatic spike was still 88 mV, measured from resting; Figure was taken after the start of perfusion with normal saline agai and shows a partial recovery of the axon spike and Figure shows the final recovery of the axon spike to 88 mV, measure from resting.

To further test the axon's dependency on sodium we treate the preparation with 30 µM tetrodotoxin (TTX: Calbiochem

TTX has been shown to block selectively the active sodium conductance in a number of excitable membranes8, and would be expected to block the axon spike if it were mainly dependent on sodium. After 10-15 min, TTX completely and reversibly blocked the axon spike in response to somatic stimulation, while having little effect on the soma spike.

If the axon membrane has a calcium channel like that in the soma, then it should be possible to produce axon spikes in a solution in which all the sodium is replaced with calcium (the somatic action potential is larger in such a solution than in normalsaline). Figure 1e-h, shows that we could not produce axon spikes in high-Ca solution by somatic stimulation. Figure 1e was obtained in normal saline, and shows two superimposed sweeps, one with a subthreshold stimulus. Figure 1f shows the blockage of the axon spike after about 2 min in Na-free, Ca 340 mM solution, while the soma spike was 108 mV. Figure 1g shows a partial recovery after the start of perfusion with normal saline, and Figure 1h shows the amount of recovery about 5 min

These experiments demonstrated that the axon could not conduct action potentials in Na-free solutions or in the presence of TTX. It was possible, however, that the axon membrane might still be excitable in Na-free solution, if a depolarising stimulus were applied close enough to the recording area to affect it directly. To test this possibility we stimulated the axon through a suction electrode applied to the cut end of the right connective. with a chlorided silver wire just outside the end of the electrode serving as the return path for the current. The recording electrode was placed in the axon at about 0.5 cm from the suction electrode. With this method of stimulation the axon spike was again blocked by replacement of external sodium with Tris. Within 5 min after perfusion with Na-free solution, depolarisations of up to 30 mV produced no regenerative response. Partial recovery of the spike was seen upon reperfusion with normal saline. The antidromic axon spike was also blocked by 30 µM tetrodotoxin in the external solution. To test the possibility that the axon membrane might have a calcium channel like that in the soma, we repeated the high-calcium experiment shown in Fig. 1e-h, with direct axonal stimulation using a suction electrode. Replacement of external sodium with calcium completely blocked the axon spike within 5 min. This experiment indicated that, when external sodium was replaced with Tris or calcium, the R2 axon was not only incapable of conduction, but was also inexcitable even with direct stimulation.

The absence of an axon spike in Na-free solution when the soma was stimulated (Fig. 1b and f) could have been due to conduction block in the axon. The partial spike seen upon reperfusion with normal saline (Fig. 1c and g), however, indicated that the amplitude of the axon spike when present was strongly dependent on external sodium. The blockage of the axon spike when external sodium was replaced with calcium showed that the axon membrane does not have a specialised calcium channel like the soma4-6. In addition these experiments suggested that no large calcium current goes through the axonal sodium channel during the action potential9.

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#### Regulation of catecholamine synthesis in the rat brain in vitro by cyclic AMP

EVIDENCE that catecholamines stimulate the production of adenosine cyclic-3',5'-monophosphate (cyclic AMP) in the pineal gland<sup>1</sup>, superior cervical ganglia<sup>2</sup> and both in brain slices3,4 and homogenates5 has suggested that cyclic AMP has an important postsynaptic function in both peripheral and central adrenergic neurotransmission. Evidence has also been presented in the hypogastric nerve-vas deferens preparation<sup>6</sup> and adrenal medulla7 that dibutyryl cyclic AMP increases the release of noradrenaline, suggesting a presynaptic role for cyclic AMP in modulating catecholamine metabolism in adrenergic terminals. In the central nervous system, adenylate cyclase and phosphodiesterase are, to a considerable extent, associated with the isolated nerve-ending (synaptosomal) fraction<sup>8</sup> in which putative neurotransmitters are primarily localised. Thus it was of interest to determine whether cyclic AMP might also stimulate catecholamine turnover in the brain, especially in synaptosomes prepared from brain homogenates.

Recent experimental evidence demonstrates that dibutyryl cyclic AMP can increase the formation of dopamine from labelled tyrosine when incubated with brain slices9 or synaptosomes<sup>10,11</sup> prepared from the corpus striatum. We report here, a stimulation of tyrosine hydroxylase activity in rat-brain synaptosomes incubated with cyclic AMP derivatives; this effect can also be produced by cyclic AMP in a soluble preparation of tyrosine hydroxylase and is associated with changes in the kinetic parameters of tyrosine hydroxylation suggestive of an allosteric activation of the rate-limiting enzyme in catecholamine synthesis.

Tyrosine hydroxylase activity was assayed in crude synaptosomal fractions of rat striatal homogenates by a procedure (Table 1) which utilises the natural endogenous pteridine cofactor of tyrosine hydroxylase12. The rate of evolution of labelled CO<sub>2</sub> resulting from the decarboxylation of newly formed 14C-dopa was linear for at least 20 min and varied directly with the quantity of tissue present in the assay. The

Table 1 Effects of cyclic nucleotides on tyrosine hydroxylase activity in striatal homogenates

Compound added	% of control
	tyrosine hydroxylase activity $\pm$ s.e.m.
8-Bromo-cyclic AMP	183 + 7*
Dibutyryl cyclic AMP	158 + 3*
Monobutyryl cyclic AMP	$152 \pm 2*$
8-Methyl-thio-cyclic AMP	$136 \pm 4*$
Cyclic AMP†	79 + 3*
Dibutyryl cyclic GMP	84 1*
Na butyrate	99 主 3

Tyrosine hydroxylase activity was determined in a preparation of striatal synaptosomes<sup>12</sup> preincubated for 5 min in the presence or absence of cyclic nucleotides (1 mM) or Na butyrate (2 mM) and incubated for a further 20 min with a saturating concentration of 1- $^{14}$ C-L-tyrosine (20  $\mu$ M), in a Krebs-Ringer-phosphate medium at 37° C, pH 6.7. Mean control activity  $\pm$  s.e.m. = 20.3  $\pm$  0.3 nmol  $^{14}\text{CO}_2$  per g wet weight h<sup>-1</sup> and is represented as 100%. \*Significant difference by Student's t test (P < 0.01) of the difference

between enzyme activity in control compared with treated tissues for at least 9 to 12 experiments.

†In the presence or absence of theophylline (1.0 mM).

activity was stereospecific for the L-isomer of tyrosine and could be inhibited by low concentrations of 3-iodotyrosine or of dopamine, or pretreatment with intracranially administered 6-hydroxydopamine. Protein was determined by the method of Lowry et al.<sup>13</sup> with bovine serum albumin as a standard.

Table 1 shows that incubation of striatal homogenates with either dibutyryl or monobutyryl cyclic AMP, which are poor substrates for phosphodiesterase and to which membranes are relatively permeable, produced similar stimulation of activity, while cyclic AMP, which is less able to penetrate cell membranes14, produced a small inhibition, even when coincubated with the phosphodiesterase inhibitor theophylline (1 mM). Enhancement of the tyrosine hydroxylase activity was also found with 8-bromo-cyclic AMP and 8-methyl-thio-cyclic AMP, further suggesting that the stimulation might be mediated by the cyclic AMP moiety of these derivatives. The stimulation of tyrosine hydroxylase activity induced by dibutyryl cyclic AMP was not due to the butyrate moiety since incubation with sodium butyrate had no effect, and a small decrease was produced with dibutyryl cyclic GMP. The stimulatory effect of dibutyryl cyclic AMP did not occur distal to the tyrosine hydroxylation step since there was no alteration in the rate of formation of <sup>14</sup>CO<sub>2</sub> when carboxy-<sup>14</sup>C-D,L-dopa (0.1 mM) was substituted for tyrosine. When the concentration of dibutyry cyclic AMP in the incubation medium was altered, significant dose-dependent increases of tyrosine hydroxylase activity were observed, from 50 µM (about 14%) to a maximum (60%) at 1.0 mM of cyclic nucleotide when striatal synaptosomes were used. The hydroxylase activities in P2 synaptosomal fractions<sup>12,15</sup>, prepared from various brain regions were measured in the presence of dibutyryl cyclic AMP (1 mM); the hydroxylation of tyrosine was increased to a similar extent in striatal (57  $\pm$  3%) and cerebral cortical (51  $\pm$  2%) synaptosomes, and an even greater stimulation occurred in the brainstem (85  $\pm$ 4%). Thus, dibutyryl cyclic AMP seems to stimulate the rate of catecholamine synthesis in isolated nerve endings in several regions of the rat brain.

Table 2 Effects of cyclic AMP on the kinetic parameters of soluble, striatal tyrosine hydroxylase

	$K_m$ -DMPH <sub>4</sub>	$K_i$ -dopamine
Control Cyclic AMP (0.01 mM)†	$0.62 \pm 0.05 \\ 0.08 \pm 0.02*$	$(mM)$ $0.09 \pm 0.02$ $0.64 \pm 0.04*$

According to the assay procedure described in Fig. 1, the  $K_m$  for DMPH<sub>4</sub> was determined by the method of Lineweaver and Burke<sup>18</sup>, at a tyrosine concentration of 0.1 mM and six DMPH<sub>4</sub> concentrations ranging from  $10^{-5}$  to  $10^{-3}$  M. Each value is the mean  $\pm$  s.e.m. of the intercepts generated from six separate lines. The  $K_i$  was determined by the method of Dixon<sup>19</sup>, at three DMPH<sub>4</sub> concentrations of  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$  M, a constant L-tyrosine concentration of  $10^{-4}$  M and seven dopamine concentrations ranging from  $10^{-5}$  to  $10^{-3}$  M.

\*Indicates significant difference from controls at P < 0.01. †Cyclic AMP was added to the assay mixture 5 min before the initiation of the reaction by addition of substrate L-tyrosine.

Since cyclic AMP derivatives might cause an alteration in membrane permeability of neural tissues, we attempted to determine whether the observed stimulation of synthesis could be explained by an alteration of the uptake of substrate tyrosine into synaptosomes. This is probably not the case, since the uptake (0.5 to 20 min) of labelled tyrosine (20 µM) into striatal synaptosomes, selectively recovered on Millipore filters<sup>16</sup>, was not altered by incubating with dibutyryl cyclic AMP (up to 5 mM). Furthermore, in synaptosomes preincubated with dibutyryl cyclic AMP and subsequently washed, tyrosine hydroxylase activity was stimulated, even though the dibutyryl cyclic AMP was not present in the incubation medium. These findings suggest that the stimulation of tyrosine hydroxylase activity is not a result of an increased uptake of labelled precursor.

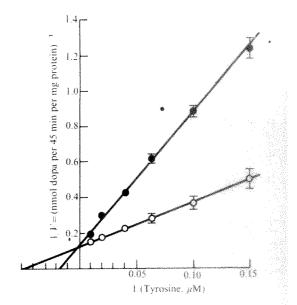


Fig. 1 Effect of cyclic AMP on the  $K_m$  for tyrosine of striat tyrosine hydroxylase. Soluble tyrosine hydroxylase activity w assayed in a 100,000g supernatant of striatal homogenates by modification of the method of Shiman et al. <sup>17</sup>. Tissues (50-70 protein) were pre-incubated for 5 min in the presence or absem of cyclic AMP (10  $\mu$ M) and incubated for a further 45 min 1.0 ml of medium containing: DMPH<sub>4</sub> (1 mM), sheep liveridine reductase, NADPH (1 mM), catalase (1,100 L sodium acetate buffer, pH 6.0, and various concentrations <sup>13</sup>H-3,5-L-tyrosine. Tyrosine hydroxylase activity was linear with time and protein concentration under the conditions used for the determination of  $K_m$ . Each value is the mean  $\pm$  s.e.m. of at least determinations. The lines drawn giving the best fit product an apparent  $K_m$  for tyrosine in cyclic AMP-treated tissues <sup>12</sup>22.3  $\pm$  2.7  $\mu$ M, which is significantly different (P < 0.01) frountreated controls of 53.6  $\pm$  3.9  $\mu$ M, with linear regressic coefficients r > 0.95.  $\bigcirc$ , 10  $\mu$ M cyclic AMP;  $\bigcirc$  control.

Another possibility would be a direct effect of cyclic A derivatives on tyrosine hydroxylase which could involve en an increased affinity for tyrosine or the pteridine cofactor, a decreased affinity for end-product catecholamines. We t investigated the effects of dibutyryl cyclic AMP and cyclic A on the activity of soluble tyrosine hydroxylase prepared fi the rat striatum in incubations which included a parti purified sheep liver pteridine reductase system<sup>27</sup> and subsatu ing concentrations of both 3, 5-3H-L-tyrosine (10 p and 2-amino-4-hydroxy-5,6,7,8-tetrahydropteridine (DMP) (0.1 mM). Activity of soluble tyrosine hydroxylase increased by about 40% and 57% with 0.1 mM dibuty cyclic AMP and cyclic AMP, respectively, while sodi butyrate had no effect. This activation was no longer evide however, when saturating concentrations of tyrosine a cofactor were used. By Lineweaver-Burke analysis18, apparent  $K_m$  for tyrosine was significantly decreased in presence of 10 µM cyclic AMP (Fig. 1) without any change  $V_{\text{max}}$ . Finally, when we applied Dixon kinetic analysis<sup>18</sup> seven-fold increase in the inhibitory constant  $(K_i)$  for dopam was observed with cyclic AMP (Table 2), suggesting a decrea affinity of soluble tyrosine hydroxylase for dopamine. In presence of cyclic AMP, a decrease in the  $K_m$  for DMP (Table 2) of seven to eightfold was also obtained withou significant change in  $V_{\text{max}}$ . A possible interpretation of the findings with soluble tyrosine hydroxylase is that cyclic Al produces an allosteric activation of striatal tyrosine hydroxyl which is mediated by increased affinities of the enzyme substrate (tyrosine) and cofactor and a decreased affinity for end-product inhibitor dopamine.

The significance of such a mechanism in the regulation catecholamine synthesis *in vivo* requires further investigation Cyclic AMP, however, may play a role in the activation tyrosine hydroxylase activity observed during increased activity

in both peripheral and central adrenergic neurones. Recent experiments have demonstrated that electrical stimulation of the guinea pig hypogastric nerve-vas deferens preparation<sup>20</sup>, as well as central dopaminergic21 and noradrenergic neurones22, causes an allosteric activation of tyrosine hydroxlase. This activation seems to be mediated by an increased affinity for both substrate and pteridine cofactor and a decreased affinity for end-product inhibitor<sup>20-22</sup> which is similar to that observed for cyclic AMP in the present experiments. In view of this theory and the observations that both electrical stimulation and depolarising agents can produce increased concentrations of cyclic AMP in brain slices23, one might postulate that cyclic AMP is partly responsible for an allosteric activation of tyrosine hydroxylase during neuronal depolarisation. Finally, the possibility that the mechanism underlying this allosteric effect involves activation of a cyclic AMP-dependent protein kinase and subsequent phosphorylation of tyrosine hydroxylase or some activator of this enzyme is under investigation.

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#### Cadaverine in the brain of axenic mice

CADAVERINE has been shown to be present in the central nervous system (CNS) (refs 1, 2 and personal communication from M. Wiechmann). Its concentration in the whole brain varies during behavioural sleep in mammals and during hibernation in molluscs1.2. It is a precursor of piperidine, which has been linked with some functional stages of the CNS. Cadaverine is the source of much of the piperidine excreted in the urine3. Cadaverine has been considered a non-metabolite of the brain, and its presence in the body was believed to be due almost entirely to bacterial decarboxylation of lysine in the intestine4. In slices of mouse brain, exogenous cadaverine accumulates against a concentration gradient and can reach a concentration ten times greater in the tissue than in the surrounding medium4. The question has been therefore: does the cadaverine in the brain originate from an exogenous source; is it formed and resorbed in the intestine? Assaying cadaverine in axenic mice, we have now found that this is not the case, and that there is an endogenous source of cadaverine in the mouse.

Ten 3-month-old mice of each of the following strains were used: C57BL/6 (pathogen free colony of this department); •CD-1 COBS (Caesarean originated barrier sustained, Charles River), and CD-1 germ-free (Charles River). For the final 14 d, the CD-1 COBS mice were fed the same diet as the germfree stock. All animals were killed when active, that is moving in the cage, grooming and so on.

The mice were decapitated, the whole brain and blood samples were homogenised in perchloric acid, and the homogenate was dansylated and separated by thin-layer chromatography on silica gel. DANS-piperidine and bis-DANScadaverine TLC fractions were identified mass spectrometrically by their molecular ions (m/e 318 and m/e 568) and quantified by comparing their integrated ion currents with those of added internal standards of DANS-pyrrolidine (m/e 304) and bis-DANS-hexamethylenediamine (m/e 582) (for details and discussion of the method see refs 2 and 5).

Table 1 summarises our results. In all three groups of mice, cadaverine was found in both the brain and blood. The differences among individual mean values were not significant. The concentrations were measured as quantities more than 10 times higher than their blanks.

Table 1 Concentrations of cadaverine in the whole brain and blood of pathogen-free, COBS and germ-free mice

Mouse strain	Brain	Blood
C57BL/6	$0.30 \pm 0.07$ (7)	$0.24 \pm 0.07$ (7)
CD-1 COBS	$0.20\pm0.06$ (10)	$0.22 \pm 0.06$ (10)
CD-1 Axenic	$0.55 \pm 0.17$ (10)	$0.27 \pm 0.12$ (10)

Figures are in  $10^{-12}$  mol mg<sup>-1</sup> wet tissue, mean  $\pm$  standard error. The numbers of measurements are given in parentheses.

The different origins and environmental conditions of tested animals had no apparent effect on the concentrations of cadaverine in their brain and blood. The lack of microbial flora in the germ-free mice also had no apparent effect on their brain and blood cadaverine levels. In contrast, there was significantly more piperidine in the blood of COBS mice  $(1.10\pm0.23~{\rm pmol~mg^{-1}})$  than in that of germ-free mice  $(0.19\pm$ 0.05 pmol mg<sup>-1</sup>; P < 0.01). It seems therefore that the cadaverine concentration in both the brain and blood of active mice is kept constant, irrespective of the formation of cadaverine by bacteria in the intestine. The increased supply of exogenous cadaverine is compensated for by its conversion to piperidine, without allowing any appreciable increase in cadaverine in brain or blood.

From our findings we make the following conclusions. (1) There is a source of cadaverine in mice that is independent of the bacterial decarboxylation of lysine in the intestine; (2) both the blood and brain concentrations of cadaverine in active mice seem to be tightly controlled and are maintained at a level which does not depend on inflow of cadaverine from the intestine and (3) the concentration of cadaverine in the blood is probably maintained by the conversion of cadaverine into piperidine.

It seems therefore that there are two pools of cadaverine in mice. One provides both the blood and brain with an amount which might have some physiological importance which is unknown at present. The other pool, originating from bacterial decomposition of the food in the intestine, seems to be efficiently transformed into other compounds, one of them piperidine. and eliminated from the body.

There are indications that piperidine might be involved in functions of the central nervous system, particularly hibernation and sleep. Its concentration in the brain and blood varies during hibernation and behavioural sleep<sup>6-8</sup>, and it has relatively strong inhibitory effects on certain molluscan cholinergic neurones\*. Our results suggest that cadaverine in the brain and blood of mice could participate in this process.

It is interesting in this context that the so called 'pink spots' observed on chromatograms of the urine of schizophrenic patients were considered to be due to the presence of monoacetyl- and monopropionylcadaverine<sup>10</sup>. If our findings with axenic mice are relevant for humans, the presence of large amounts of anomalous metabolites of cadaverine in the urine could reflect not only the altered dietary conditions of schizophrenic patients as suggested10, but may also indicate a failure of the normal catabolism of cadaverine in their central nervous system.

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#### Foetal origin of transferrin in mouse amniotic fluid

THE measurement of specific foetal products in amniotic fluid collected after amniocentesis offers an obvious approach to the foetus in utero. The proteins in mouse

amniotic fluid consist of three transferrins (Trf1, 2 at 3), five  $\alpha$  foetoproteins ( $\alpha$  FP 1-5) and albumins, the pr portions of which change during gestation1,3. The change in composition directly parallel those observed in foet serum, except that they occur 24 h later in the amnio fluid. No comparable alteration is observed in matern serum patterns. Transferrin 3 constitutes the major tran ferrin band both in late-stage foctuses and in adults, as so could be derived from either the foetus or the mothe We have investigated the origin of transerrin 3 in mou amniotic fluid, using a genetic difference at the transferr locus3 as a marker.

Reciprocal embryo transfers were made between inbr C3H/Bi/McL and randomly bred O-strain mice Blast cysts were flushed from donor uteri 3.5 d post coitum, at injected into the uteri of pseudopregnant recipients 2.5 post coitum4. Plasma was collected from donor females ( severing the carotid arteries) at transfer and from recipie females when autopsied on day 18 of gestation. Foetus were dissected from the uterus, with embryonic membran intact, and amniotic fluid sampled by insertion of 26-gauge needle directly into the amniotic cavity. Foet plasma was collected in heparinised capillary tubes fro an incision made in the external carotids. Fluids we analysed for protein on polyacrylamide slab gel electr phoresis'. Protein concentration was determined with the Lowry6 technique, using bovine serum albumin as standar

All Q donors and recipients proved to be Trfb/Trfb, at C3H Trf\*/Trf\*. Analyses were carried out on six C3 embryos on Q recipients, and three Q embryos in C3 recipients, and the transferrin type of the foetus provi in every case to resemble that of the donor strain (Fi 1A). Amniotic fluid transferring were identical in electrons phoretic mobility to those of the foetuses with which the were associated. Mixtures of plasma of recipient femal and transferred foetuses or their amniotic fluids resolve two Trf 3 bands, while mixtures of the donor plasm with foetal plasmas or amniotic fluids yielded one on (Fig. 1B), clearly demonstrating the foetal origin of the Trf 3 transferrin in the amniotic cavity.

Since Gustine and Zimmerman have already shown foetal origin of the five  $\alpha$  foetoproteins and Trfs 1 and our result indicates that all mouse amniotic fluid protein with the possible exception of the albumins, are of foet origin. The similarities between the protein patterns amniotic fluid and foetal plasmas, and the absence of mar maternal-type proteins in either fluid, support this. Th yolk sac, which is one site of  $\alpha$  foetoprotein synthesis completely surrounds the amnion in the mouse, and ma be important for transfer of the other proteins as well, not for their synthesis. The increase in sialyl transferal activity in the foetal liver coincides with the increase number of sialic acid residues in the foetal plasma foetoprotein, while the comparable changes in yolk sa sialyl transferase activity and in amniotic fluid a foet protein occur about one day later<sup>1,7</sup>. This suggests that the α foetoprotein in foetal plasma may be mainly derive from the foetal liver and the a foetoprotein in amniot fluid may be derived from the yolk sac.

The rapid changes in the volume and protein pattern of mouse amniotic fluid<sup>1,2,8</sup> may be related to the sho time spent in the uterus. Indeed, mouse amniotic flus perhaps by virtue of its proximity to the large and va cular yolk sac, has considerable similarities to marsupi yolk sac fluid, which is believed to play an important ro in foetal nutrition9, and in which, like the mouse amniot fluid, the transferrins are of foetal origin 16. Although the placental attachments are quite different in mice an marsupials, in both species the yolk sac prevents the amnion from making contact with the uterine lumen an maternal circulation, unlike in man, where the amnion in close contact with the uterine epithelium.

The obvious route by which a transferrin of foetal origin could pass into the amniotic cavity in mice is from the vitelline vessels across the exocoel and amniotic membrane (Fig. 2). It seems unlikely that transferrin proteins would diffuse from the foetal skin or alimentary canal into the amniotic cavity and the area around the umbilical stalk where the amnion is close to the allantoic vessels is small. In man, however, these other, perhaps less efficient, routes are the only ones available for transfer of foetal protein to the amniotic cavity, as the yolk sac remains small and rudimentary throughout development.

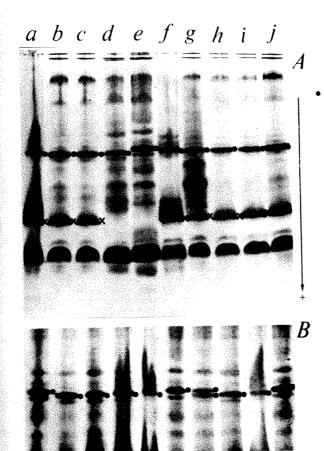


Fig. 1 Electropherogram of mouse plasmas and amniotic fluid. A, Replications A and B: C3H blastocysts (Trf\*/Trf\*) transferred to Q recipients (Trf\*/Trf\*). Wells from left to right: a, amniotic fluid; b, foetal plasma 1; c, foetal plasma 2; d, C3H donor plasma; e, Q recipient plasma; f, amniotic fluid; g, foetal plasma 1; h, foetal plasma 2; i, foetal plasma 3; j, foetal plasma 4. B, Mixtures of donor and recipient plasmas and amniotic fluid. Wells from left to right: a, recipient plasma Q (Trf\*/Trf\*); b, foetal plasma (C3H)+donor plasma (C3H); c, donor plasma C3H (Trf\*/Trf\*); d, amniotic fluid+donor plasma; e, amniotic fluid; f, recipient plasma; g, recipient+donor plasma; h, donor plasma; i, amniotic fluid; j, recipient plasma. Note that the only mix which yields two transferrins is that of donor and recipient plasmas: donor plasma mixed with its transferred recipient plasmas: donor plasma mixed with its transferred foetal plasma or amniotic fluid gives only one transferrin. Gels were run at 30 mA, 100 V for 2.5 h, and stained in 0.025% Coomassie Blue+0.1% Amido Black in 25% methanol and 7% acetic acid for 45 min. Destaining was by diffusion for 3 h in 25% methanol and 7% acetic acid in water. 25 µl of amniotic fluid (3 mg ml<sup>-1</sup>) and 2.5 µl of foetal plasma (20 mg ml<sup>-1</sup>) and maternal plasma (45 mg ml<sup>-1</sup>) were applied to each well. A tracking dye of bromophenol blue in 40% sucrose was used to colour the samples and show the boundary of the fastest moving components. The extra recipient plasmas: donor plasma mixed with its transferred the boundary of the fastest moving components. The extra bands visible in the  $\beta$  globulin region (between transferrin and a foctoprotein) in some samples are due to free haemo-globin and haemoglobin/haptoglobin (Hb/Hp) bands resulting from haemolysis of those plasmas during collection. •, Transferrin proteins;  $\times$ ,  $\alpha$  foetoproteins.

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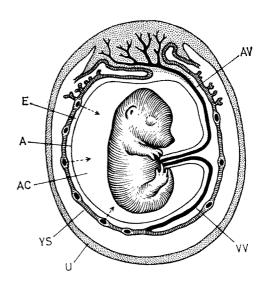


Fig. 2 The arrangement of foetal membranes in the mouse on day 15 of gestation, showing the likely route of transfer (arrows) of protein from the foetal circulation to the amniotic fluid. The orientation and relative proportions of the embryo are diagrammatic. E, Exocoelomic cavity; A, amnion; AC, amniotic cavity; VV, vitelline vessels; AV, allantoic vessels; YS, yolk sac splanchnopleur; U, uterus.

The composition and origin of human amniotic fluid components reflect this difference. Group-specific component and transferrin, unlike those in the mouse, have been shown to be of maternal origin<sup>11-14</sup>, and the relative proportions of different serum proteins in amniotic fluid throughout pregnancy resemble those in maternal serum11. Although the foetus makes some contribution13,16, the only proteins for which a foetal origin has been unequivocally demonstrated are hexosaminidase A and B, arylsulphatase A,  $\beta$  galactosidase and  $\alpha$  foetoprotein<sup>17-21</sup>. In man, the relative concentration of a foetoprotein in amniotic fluid and in foetal serum is 1:150 between 10 and 20 weeks11, whereas in the mouse it is approximately 1:10 at days 11-18. Clearly, the foetal contribution to amniotic fluid is more substantial in mice than man.

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#### Liposomes can mimic virus membranes

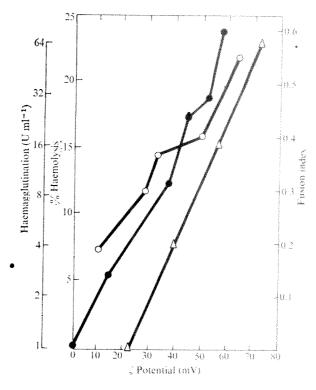
Considerable insight into mechanisms of membrane permeability and transport has been obtained from the study of simple lipid membranes. For the study of membrane-membrane interactions, however, such model systems have been used to a much lesser extent. If present ideas that biological membranes possess extended areas of lipid bilayer are correct, then it is probable that bilayer regions are involved in intermembrane phenomena such as adhesion and fusion, and it becomes reasonable to use simple lipid membranes to mimic such behaviour.

We set out to do this with respect to the membrane associated activities exhibited by many of the enveloped viruses. As the model virus, we used a modification of what has been described as a lipid dispersion, lipid vesicles, or liposomes, that is, single or multiple concentric shells of lipid bimolecular leaflets. The 'non-genetic' viral activities that are duplicated by the model virus membrane include attachment of the virus to the host cell. fusion of the viral membrane with the cytoplasmic membrane of the host cell, fusion of membranes of two or more host cells, and in the case of erythrocytes, haemolysis.

With respect to cell fusion, lysolecithin alone and in an oil emulsion has been used with success, although cell lysis is a problem<sup>1,2</sup>. Very recently, mixtures of phosphatidyl choline and either phosphatidyl serine (PS) or phosphatidyl glycerol in the form of liposome dispersions have been shown to be effective without adverse influence on cell viability<sup>3</sup>. In a related study, it was shown that lipid from phospholipid vesicles is incorporated into mycoplasma membrane, although the mechanism was not unequivocally elucidated4.

Lipid vesicles were prepared in the usual manner<sup>5</sup>. Lipids were dissolved in chloroform-methanol (4:1) which was subsequently removed under reduced pressure. The dry lipids were suspended at 2.5 mg ml<sup>-1</sup> in sucrose (0.3 M), EDTA  $(10^{-4} \text{ M})$  and tricine  $(2.0 \times 10^{-2} \text{ M})$  at pH 7.8 (stock lipid dispersion). Liposomes used in haemolysis experiments were then sonicated with a probe type apparatus for a few minutes at room temperature. Vesicles used in fusion experiments were sonicated further (40 min at 24° C) to yield a population with a high proportion of unilamellar vesicles<sup>6</sup>. Haemagglutination, haemolysis and cell fusion were assayed using standard virological methods. The zeta potential of liposomes was determined by microelectrophoresis7. The lecithin used in this study, diisostearoylphosphatidyl choline (L), was prepared by reaction of the appropriate anhydride with glycerophosphoryl choline in the presence of triethylisostearate\*. Lysophosphatidyl choline (LL) was prepared from this lecithin by hydrolysis with phospholipase A (ref. 9) and purified by ether precipitation. Stearylamine (SA, 99.5%) was purchased from Lachat Chemical

Figure 1 depicts the relationship between the zeta potential of liposomes containing up to 2% SA and the haemagglutination



Membrane activities are related to liposome surface O, Okada's fusion index for EATC<sup>16</sup> for liposomes containing 25% LL and various amounts of SA (0.2-3.0%). Haemolysis of sheep red cells by liposomes containing 20. LL and varying amounts of SA. △, Log<sub>10</sub> of the haemagglutination titre of liposomes (5 mg ml<sup>-1</sup>) containing only lecithin and SA. All functions are plotted against the zeta potential of the corresponding liposomes. The haemagglutination titre of lipo some dispersions was measured by the Salk pattern method13 using a 0.5 % (v/v) suspension of washed sheep red blood cells in 0.3 M sucrose buffered with 20 mM tricine (pH 7.6). For haemolysis experiments, equal volumes of a 10% suspension of the sheep cells in buffered sucrose and the stock liposome dispersion were mixed at 4° C and the cells were allowed to The temperature was then raised to 37° C. After agglutinate. 30 min, % haemolysis was determined by the method of Neurath and Sokol12. Ehrlich ascites tumour cells for fusion experiments (EATC) were grown intraperitoneally in adult Swiss mice of both The cells were then washed three times with Hank's balanced salt solution (BSS) to remove contaminating crythrocytes and suspended at a concentration of 10 % (v/v) in 0.3 M sucrose buffered to pH 7.8 with 20 mM tricine-NaOH. 0.25 ml of lipid dispersion was added to 1 ml of a 10% EATC suspension. The incubation procedure for fusion was the same as that used in the haemolysis experiments. Fusion was assayed using methods described by Okada<sup>16</sup>

(HA) titre of a dispersion containing 5 mg ml<sup>-1</sup> lipid. When plotted on a logarithmic unit scale, the relationship is linear up to 70 mV, above which value it would no longer be expected that the zeta potential would be an accurate measure of the surface potential. That the liposomes are the actual agglutinating agent rather than unincorporated SA is indicated by the following observations. First, when such dispersions are centrifuged at speeds sufficient to sediment liposomes but not SA micelles, 99% of the HA activity is found in the pellet. Second, the zeta potential of liposomes is proportional to the SA content and is stable for hours, indicating that there is little tendency for the SA to leave the liposomes.

Inclusion of LL into positively charged liposomes renders them active in the lysis of sheep erythrocytes. At constant surface charge density, the haemolysis rate increases with increasing LL content. Similarly, at constant LL values, the extent of haemolysis increases linearly with zeta potential. This is illustrated in Fig. 1. Neutral and negatively charged vesicles are devoid of haemolytic activity at the concentrations tested. We have compared the time course of liposome-induced haemolysis with that which has been reported for EDTAtreated Sendai virus and, aside from a scaling factor, the relationships are virtually identical. In addition, there is a linear relationship between the concentration of liposomes (HA titre) and the logarithm of percentage haemolysis, the same kind of relationship that obtains with EDTA-treated Sendai virus<sup>13</sup>.

Liposomes capable of haemolysis also promote fusion of actively metabolising cells. Two animal cell types, EATC and KB, exhibit extensive cell fusion following treatment of cultures with L-LL-SA liposomes. Micrographs of representative examples of polykaryons of such cells are shown in Fig. 2. In our experience polykaryons produced by liposomes and Sendai virus are indistinguishable. As is the case with the fusion-promoting viruses, suspended cells treated with liposomes agglutinate at 4° C and begin to fuse as the temperature is raised. The fusion index for EATC increases with both LL content and positive surface potential of the liposomes. The latter relationship is shown in Fig. 1, the data in which represent fusion indices determined shortly (30 min) after liposome treatment. Although, as is the case with virus-induced fusion, there is some loss of cell viability, it is rather minimal. For example, 30 h after a 15 min treatment with liposomes containing 20% LL and 5% SA, fusion indices were 45±5% for KB and rabbit kidney cells and 0.58 (Okada's index for cells in

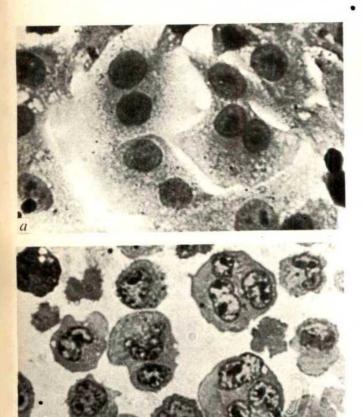


Fig. 2 Liposome-induced cell fusion. Polynuclear cells formed following treatment of a, KB cells monolayer and b, Ehrlich ascites tumour cells in suspension, with liposomes containing 20% LL, 5% SA and 75% L. 0.25 ml lipid dispersion was added to 1 ml of a 10% EATC suspension or to a KB monolayer in a 25 cm² culture flask containing 5 ml BSS. KB cells were grown in Eagle's minimal medium (EMM) and supplemented with 20% heat inactivated foetal calf serum in 25 cm² plastic flasks. The cultures were grown to confluence and maintained in EMM supplemented with 5% calf serum. For fusion experiments, the monolayers were washed with prewarmed BSS and treated with 0.25 ml of the liposome suspension at 37° C for 15 m. The cultures were then washed with BSS to remove excess liposomes and fresh maintenance medium added. For fusion assays, the cells were washed with BSS, treated with 0.02% EDTA in PBS for 1 min, fixed with 2% glutaraldehyde, stained with May-Greenwald Giemsa stain and polynuclear cells counted as described by Kohn<sup>14</sup>. EATC were treated as described in the legend to Fig. 1.

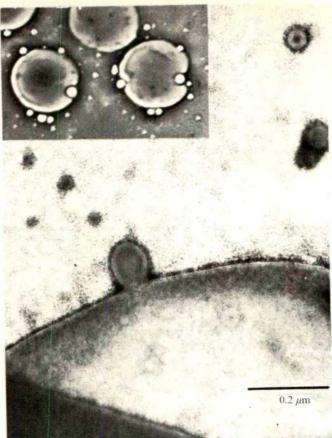


Fig. 3 Electron micrograph of a negatively stained preparation of human red cells treated with liposomes. A liposome is seen fusing with the erythrocyte membrane (Bar represents 0.1 μm). The inset is a phase micrograph of liposomes of the same composition adhering to the surface of red cells (×900). Freshly drawn human group O erythrocytes were washed five times with PBS and suspended at a concentration of 50% (v/v) in 0.3 M sucrose containing 0.1 M phosphate buffer (pH 7.3). The cells were then treated with unsonicated liposomes containing 20% LL, 5% SA and 75% L for 5 min at 37° C. A drop of the cell suspension was then lysed at the air-water interface and red cell ghosts floating on the surface were picked up on carbon strengthened parlodion grids. Without letting the preparation dry, the cells were fixed in osmium vapour for 5 min. The excess water was then removed from the grid surface with blotting paper and a drop of 2 potassium phosphotungstate (pH 6.5) was immediately placed on the grid. After 30 s the excess stain was removed with blotting paper and the preparation was allowed to air dry. The grids were then examined in a Hitachi 11-A electron microscope. For light microscopy, grids containing unstained liposome-treated ghosts were examined directly under the phase microscope.

suspension) for EATC. In all cases about 10% of the cells died (trypan blue exclusion) or detached from the substrate. This degree of cell loss accompanies each treatment; the extent of fusion, however, can be increased by multiple exposures of cells to the liposomes. Cell damage can be minimised by decreasing the liposome/cell ratio. In our experiments, each cell was treated with about 10s liposomes. This amount can be reduced by four orders of magnitude, and the fusion index is lowered by only 10%. At such low liposome levels, the decrease of cell viability in fusion experiments is reduced to practically nothing. These results are similar to those reported by Papahadjopoulos et al.<sup>3</sup> for what they found to be the most efficient lipid mixture, L and PS. We have not obtained appreciable fusion with PS liposomes in our conditions.

By electron microscopy, enveloped viruses have been observed fusing with cell membranes<sup>15–19</sup>. It has been suggested that infection occurs by the same mechanism and, further, that binucleate cells are formed by the simultaneous fusion of the virus membrane with the membranes of two cells<sup>17</sup>. The fusion of liposomes with cell membranes may also be seen by electron

microscopy. Figure 3 is an electron micrograph of an L-LL-AS liposome in an intermediate stage of fusion with a human erythrocyte. A variety of such electron micrographs of cell membranes taken shortly after treatment with L-LL-SA liposomes reveals various stages from simple adhesion to nearly complete incorporation. The inset is a phase micrograph of liposomes of the same composition adhering to the surface of

The advantages of using simple artificial means to produce hybrid cells for genetic and other experiments have been noted3. The use of liposomes circumvents the necessity of finding a virus competent to fuse the cells of interest. As all cell membranes are negatively charged, the receptor for an 'artificial virus' of the type described here is universal. Model viruses also offer the opportunity of introducing foreign molecules into intact cells or into their membranes by incorporating such molecules into the aqueous or membrane phase, respectively, of the liposome20.

The data presented here show that a lipid bilayer vesicle behaves like some virus membranes when a long chain cation and a lytic lipid are incorporated into it. It is possible therefore, that the basic requirements for viral membrane proteins that participate in infection activities, are to recognise specifically the host cell as, for example, would an antibody, and to disorder bilayer membranes in a manner similar to that of lyso compounds and detergents. We do not intend to imply either that viruses are positively charged or that lysolecithin is a necessary viral membrane component.

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#### Incorporation of lipid vesicles by mammalian cells provides a potential method for modifying cell behaviour

PHOSPHOLIPID vesicles (liposomes1) have been used extensively as models for natural membranes1.2. Multilamellar vesicles are incorporated by mammalian cells both in vivo3,4 and in vitro5. Sonication of such vesicles creates small (250-500 Å diameter)

closed spherical vesicles composed of a single lipid bilayer enclosing an aqueous space2. Cultured mammalian cells will also incorporate these unilamellar vesicles without cytôtoxic effects6. We present here data on the uptake of unilamellar vesicles by cells in vitro and the use of these vesicles as carriers to introduce biologically-active molecules into specific regions of the cell.

Incorporation of unilamellar vesicles with negative or positive surface charge by mouse 3T3 and L929 cells and human erythrocyte ghosts is shown in Fig. 1. Each cell type incorporated substantial numbers of vesicles (1 nmol lipid =  $2 \times 10^{10}$ 

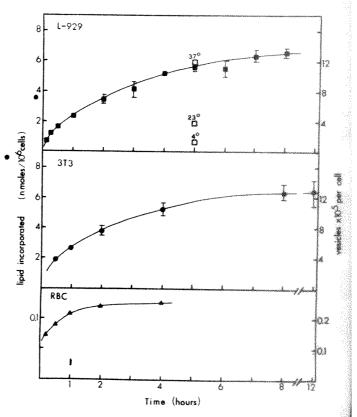


Fig. 1 Incorporation of unilamellar lipid vesicles by mouse 313 and L929 cells and human erythrocyte ghosts, at 37° C. Vesicles were prepared by dispersion and sonication of highly purified lipids in calcium and magnesium-free Hanks solution, pH 7.4, as before using 4 µmol of phospholipid in 2 ml. Preparations were centrifuged at 100,000g for 30 min before use to remove contaminating multilamellar vesicles. Lipids were synthesised and purified as before? Phosphatidylserine (PS) was from bovine brain and phosphatidylcholine (PC) from egg yolk. BALB/c mouse 3T3 cells and mouse L929 cells were cultured as before<sup>6.7</sup>. Human erythrocyte ghosts were prepared from fresh blood by the method of Jung et al.8 which yields 'resealed' ghosts with a restored permeability barrier to small solutes. 3T3 and L929 tell populations were incubated with vesicles as before. Erythrocyte ghost preparations were equilibrated in 50 mM NaCl, 2 mM histidine, 2 mM N-Tris (hydroxymethyl)-methyl-2-aminoethane sulfonic acid (TES) and 0.1 mM EDTA, pH 8.2, before incubation with vesicles. I lalex tion with vesicles. Unless stated otherwise, vesicle incorporation by cells was measured at 37° C by the uptake of 3H-dipalmitoylphosphatidylcholine (\*H-DPPC) (specific activity 4 Cimmol added in trace amounts during the formation of vescicles as described previously. L929 cell monolayers and erythrocyte ghosts in suspension were incubated with vesicles composed of PS, PC and cholesterol (1:9:8 molar ratio and trace amounts of <sup>3</sup>H-DPPC). 3T3 cell monolayers were incubated with vesicles containing stearylamine, PC and cholesterol (1:4:3 molar ratio and <sup>14</sup>C-cholesterol as a tracer). 3T3 and L929 cells were exposed to an input of 50 nmol of phospholipid per  $10^8$  cells (approximately  $1\times10^7$  vesicle per cell). Erythrocyte ghosts were treated to an input dose of 1.3 nmol of phospholipid per 10° cells. 3T3 and L929 cell monolayers were suspended by treatment with 0.2% trypsin and 0.2% EDTA in Hanks saline at intervals after incubation with vesicles and the amount of cell-associated radioactivity measured as before. ■, L929 cells incubated at 37° C; ☐, L929 cells incubated at 37° C, 23° C and 4° C as designated in the figure; ●, 3T3 cells at 37° C; ▲, erythrocyte ghosts at 37° C.

vesicles)7 but the kinetics were different in each case. For 3T3 and L929 cell cultures, the maximum incorporation of vesicles shown in Fig. 1 represents between 2 and 5% of the vesicle population, while ghosts incorporated up to 10% of the vesicle population. Dye-exclusion measurements revealed no significant change in the viability of vesicle-treated 3T3 and L929 cells compared with untreated cells and their growth rate remained comparable with that of controls during successive subcultivations for 3 months. Figure 2 shows that vesicle uptake by 3T3 cells was linear over a 10,000-fold concentration range. Vesicle incorporation by L929 cells and erythrocyte ghosts was also generally similar. Chemical analysis of the lipid content of L929 cells and erythrocyte ghosts after a 6 h incubation with phosphatidylserine (PS)/phosphatidylcholine (PC) cholesterol (1:9:8 molarratio) vesicles (conditions as in Fig. 1) revealed respectively a 20% and 15% increase in total phospholipid. Extraction of cell lipids in chloroform-methanol and thin-layer chromatography of the extracted lipids after incubation with PS/PC/cholesterol vesicles plus trace amounts of 3H-dipalmitoyl phosphatidylcholine (3H-DPPC) revealed that 70% of the incorporated 3H-DPPC label co-chromatographed with pure samples of PC, PS and phosphatidylethanolamine (PE). Tritium label was recovered from both plasma membrane and intracellular membrane fractions.

Uptake of unilamellar lipid vesicles by cultured cells was temperature-dependent (Fig. 1). Uptake of vesicles by cells was not significantly altered by inhibition of cellular energy metabolism by incubating cells for 30 min with sodium azide  $(2 \times 10^{-4})$ M) or sodium iodoacetate  $(2 \times 10^{-4} \text{ M})$  before addition of vesicles. Similarly, preincubation of cells with ouabain (10<sup>-3</sup> M) for 1 h had no effect on vesicle incorporation, and pretreatment with 10 µg ml<sup>-1</sup> polylysine (molecular weight 2,800) for 1 h to stimulate endocytosis9 also failed to alter the extent of vesicle uptake. Negatively charged (PS), neutral (PC) and positivelycharged vesicles (20% stearylamine in PC) were incorporated equally well by cells. Comparable incorporation was also found using vesicles composed of lipids that were 'fluid' (10% PS in PC) or 'solid' (10% PS in distearoylphosphatidylcholine) at 37° C. Incorporation of equimolar amounts of cholesterol into 'fluid' (PS/PC) or 'solid' (PS/DPPC) vesicles produced no substantial change in cellular uptake of vesicles. In spite of these general similarities, 'fluid' lipid vesicles can be incorporated into cells by a different pathway than 'solid' vesicles (see below).

Vesicle components could be incorporated into cells by several non-exclusive mechanisms. Exchange diffusion of lipid molecules (cholesterol) between vesicles and natural membranes

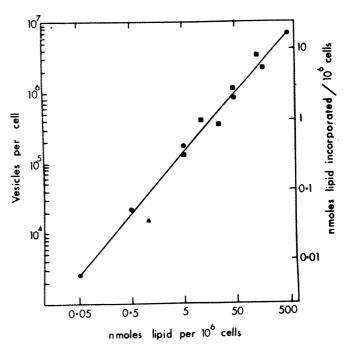


Fig. 2 Incorporation of unilamellar lipid vesicles by mouse 3T3 and L929 cells exposed to different concentrations of vesicles. 3T3 cells (●) were incubated for 4 h at 37° C with stearylamine, PC and cholesterol vesicles (1:4:3 molar ratio with ³H-DPPC as a tracer). Vesicle uptake was determined by measurement of cell-associated radioactivity as in Fig. 1. L929 cells (■) and erythrocyte ghosts (▲) were incubated for 4 h at 37° C with PS, PC and cholesterol vesicles (1:9:8 molar ratio with ³H-DPPC as a tracer).

has been documented<sup>10,11</sup>. Alternatively, vesicles can be incorporated intact by cells either by fusing with the plasma membrane or by endocytosis. Finally, the recovery of 'markers' from vesicles in association with cells might merely reflect binding of vesicles to the cell surface without incorporation *per se*. Incubation of L929 or 3T3 cells with PS/PC/cholesterol vesicles containing trace amounts of <sup>14</sup>C-cholesterol and <sup>3</sup>H-DPPC 'markers' revealed that the ratio of <sup>3</sup>H to <sup>14</sup>C counts recovered in association with cells after incubation with vesicles was similar (1:1.8) to the ratio in the original vesicle population (1:1.9), suggesting incorporation of intact vesicles. The parallel uptake of <sup>3</sup>H-DPPC and <sup>14</sup>C-cholesterol suggests tha

Table 1 Effects of cyclic	Nucleotide	Cell growth	and free cyclic nucleotides on the gro		Vesicle uptake	
Treatment	concentration (M)	as % control*	total†	nmol per 10 <sup>6</sup> cells‡	total§	nmol lipid per 10° cells¶
'Fluid' vesicles 'Fluid' vesicles containing cyclic AMP 'Solid' vesicles 'Solid' vesicles containing cyclic AMP Exogenous cyclic AMP Exogenous dibutyryl cyclic AMP	$0 \\ 10^{-5} \\ 0 \\ 10^{-5} \\ 10^{-5} \\ 10^{-5}$	108.3 25.6 105.4 97.2 94.0 70.3	4.9  4.2 0.4 NM	0.98 	5.1 5.4 4.6 4.9	2.55 2.70 2.31 2.45

Plastic Petri dishes (60 mm) were seeded with  $3 \times 10^5$  3T3 cells and incubated at  $37^{\circ}$  C for 24 h. The culture medium was then replaced with medium containing unilamellar lipid vesicles, similar vesicles containing  ${}^3$ H-cyclic AMP or medium containing  ${}^3$ H-cyclic AMP plus  $10^{-3}$  M theophylline or dibutyryl cyclic AMP plus  $10^{-3}$  M theophylline, and the number of cells were counted at intervals. Control cultures were incubated in normal medium without vesicles or cyclic nucleotides. Vesicles containing cyclic AMP were prepared as described before. The results represent mean values from three separate experiments. Fluid vesicles were stearylamine, PC and cholesterol (molar ratio 1: 4: 3); solid vesicles were stearylamine, DPPC and cholesterol (1: 4: 4, molar ratio). Both fluid and solid vesicles contained trace amounts of

\*C-cholesterol.

\*Calculated from cell counts on control and experimental cultures 6 d after inoculation.

†Cell-associated <sup>3</sup>H-cyclic AMP measured after 24 h at 37° C as a percentage of the total <sup>3</sup>H-cyclic AMP to which cells were exposed. †Calculated from values for cell-associated <sup>3</sup>H-cyclic AMP after 24 h incubation at 37° C and known values for radioactivity per µmol

SCell-associated <sup>14</sup>C-cholesterol measured after 24 h at 37° C and expressed as a percentage of the radioactivity in the original vesicle popula-

¶Calculated from the cellular incorporation of <sup>14</sup>C-cholesterol after 24 h of incubation with the vesicles and the specific activity of the cholesterol in the vesicles.

NM, not measured.

molecular exchange between cell membranes and vesicles is not the major mechanism for uptake of vesicle components since cholesterol would exchange at a faster rate10. The lack of effect of inhibitors of energy metabolism on vesicle uptake (see above) suggests that classical endocytosis is probably not involved, though incorporation of material with molecular weights lower than 2×105 by metabolically-independent endocytotic mechanisms has been described12.

We have also studied the cellular uptake of vesicles (stearylamine/PC/cholesterol; 1:4:3 molar ratio) containing 3H-cyclic adenosine 3': 5' monophosphate (3H-cyclic AMP) trapped within the internal aqueous space<sup>6</sup>. Incubation of 3T3 cells with such vesicles resulted in significant uptake of both <sup>14</sup>C-cholesterol and <sup>3</sup>H-cyclic AMP molecules from the vesicles (Table 1). Vesicle-incorporated cyclic AMP produced significantly greater inhibition of cell growth than equivalent concentrations of exogenous cyclic AMP or dibutyryl cyclic AMP plus theophylline (Table 1). Since the growth inhibition by exogenous nucleotides was similar to that reported for free nucleotides13, our results indicate that vesicles offer an efficient carrier vehicle for increasing the uptake of biologically important molecules into cells. The ratio of 3H to 14C counts recovered from vesicletreated cells was similar to that of the original vesicle population, supporting the earlier suggestion that intact vesicles are incorporated.

The alteration of cell growth by cyclic AMP-containing vesicles suggests that active cyclic AMP is released from vesicles into the cytoplasm. The simplest, but not the only possible interpretation for this presumed cytoplasmic distribution of cyclic AMP, is that some vesicles may fuse with the plasma membrane, introducing their contents directly into the cytoplasm. Using the same experimental protocol, 3H-cyclic AMP trapped within vesicles prepared from 'solid' lipids (stearylamine/DPPC/cholesterol) did not induce significant alteration in cell growth in spite of significant vesicle uptake (Table 1). This contrasting effect between 'fluid' and 'solid' vesicles could reflect differences in the 'leakiness' of the two types of vesicles for the trapped cyclic AMP. Alternatively, the vesicles may differ in their ability to fuse with the cell surface. 'Solid' lipid vesicles are unable to fuse cells7 and have a very limited capacity

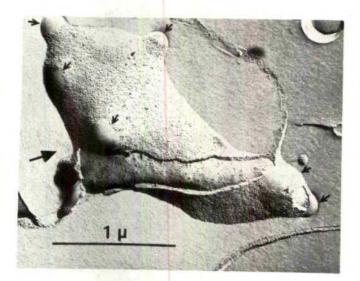


Fig. 3 Electron micrograph of freeze-fractured humanerythrocyte ghost after incubation with multilamellar lipid vesicles, showing areas (small arrows) devoid of intramembranous particles. Vesicles (PS/PC, 1:9 molar ratio) were dispersed by mechanical (vortex) agitation in 50 mM NaCl, pH 8.2. Incubation with ghosts was performed in the same buffer with 1.3 nmol phospholipid per 10<sup>6</sup> cells, for 30 min at 37° C. The cells were spun down at room temperature and resuspended in 30% (v/v) glycerol, frozen in Freon 22, fractured at -115° C in a Balzers BA360 apparatus and shadowed with platinum-carbon as before17. Large arrow indicates direction of shadowing.

to fuse with each other compared with 'fluid' vesicles14, 'Soli vesicles containing cyclic AMP might not, therefore, be able fuse with the plasma membrane and may be incorporate primarily by endocytosis, introducing their contents instead into the lysosomal apparatus. This would reduce the likelihoo of cyclic AMP reaching the cytoplasm in an active form, sin lysosomes contain several phosphodiesterases15

The incorporation of lipid molecules from 'fluid' vesicles in the plasma membrane, presumably by fusion, is further su gested by the recovery of radiolabelled lipid in the plasm membrane fraction of fractionated cells (see above) and also I changes in the lectin agglutinability, and membrane transpo properties of vesicle-treated cells (our work in preparation Figure 3 shows a freeze-fractured erythrocyte ghost aft incubation with multilamellar PS/PC vesicles. The 'ba patches' on the ghost were found in 5% of the treated cells, b were not found on several hundred fields of untreated control The dimensions and surface topography of the 'patches' a very similar to vesicles and it is tempting to interpret that the areas represent sites where vesicles have fused with the plasm membrane. Also, the density of intramembranous particles of vesicle-treated cells not showing 'patches' was significant lower than in untreated cells (our work in preparation), which suggests that the cell surface area had increased.

Our results indicate that mammalian cells can incorpora large numbers of lipid vesicles without cytotoxic effects. Fusic of vesicles with the plasma membrane may represent an impo tant pathway for vesicle incorporation, though endocytosi surface adsorption and molecular exchange between vesicle and cell membranes may also be occurring simultaneously Although the precise contribution of different pathways in the incorporation of vesicles into cells remains to be elucidated, th present results, together with other recent studies, indicate the lipid vesicles offer a potentially useful tool for modifying ce behaviour by introducing new material into the plasma men brane (ref. 16 and our work in preparation), and by the deliver of biologically active molecules and drugs into the cell3-Independent data on vesicle uptake by mammalian cells as given by Pagano et al.18 in the next communication.

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# Interaction of phospholipid vesicles with cultured mammalian cells

To delineate some of the relationships between membrane composition and the functional states of mammalian cells, we are exploring the possibility of producing controlled modifications in the plasma membrane of cultured cells, using artificially generated lipid vesicles1-3. Although other groups have reported the use of lipid dispersions for producing cellular modifications4-10, the underlying molecular mechanisms by which these effects are produced have not been determined. We present here preliminary findings on the mechanism of interaction of chemically and physically well-defined phospholipid vesicles with cultured mammalian cells.

Typical data obtained when Chinese hamster V79 cells were incubated with vesicles produced from radiolabelled egg yolk lecithin are given in Fig. 1. Unilamellar (F-II) vesicles were more efficient in the transfer of exogenous phospolipid to the cells than multilameller (F-I) vesicles. In both cases a considerable amount of phospholipid became associated with the cells in a relatively short time. The amount of lipid taken up depended on the physical state of the lipids comprising the vesicles, being substantially greater below the gel-liquid crystalline phase transition temperature. Between 20% and 30% of the labelled phospholipid taken up by vesicle-treated cells appeared in their plasma membranes. This lipid was not degraded, but remained as lecithin. The remaining radioactivity was distributed into intracellular fractions in which considerable degradation was observed. No effect on cell viability could be detected for short periods of incubation (up to 6 h), and normal cell growth resumed when the vesicle suspension was replaced by fresh medium. Prolonged incubation of sparse cultures (~103 cells cm-2), however, resulted in considerable cell death as determined by dye exclusion tests. This deleterious effect of lipid vesicle treatment agrees with our observation that sparse cultures take up about an order of magnitude more phospholipid than those approaching confluency.

Figure 2a shows a transmission electron micrograph obtained from a thin section through a preparation of V79 cells incubated with a suspension of F-I vesicles. A large vesicle, about 7,000 Å in diameter, with the characteristic multilamellar appearance of this vesicle fraction, is seen in direct contact with the plasma membrane of the cell. Figure 2b shows a preparation of unilamellar vesicles, about 500 Å in diameter, incubated with V79 cells pretreated with cationised ferritin<sup>12</sup> (0.5 mg ml<sup>-1</sup> for 5 min at 25° C). There are numerous vesicles, both attached to the surface membrane and apparently fused with the cell. Although there were no essential differences in the amount of phospholipid taken up in the presence or absence of cationised ferritin, the fate of the applied unilamellar vesicle suspension could not be traced by electron microscopy without this marker.

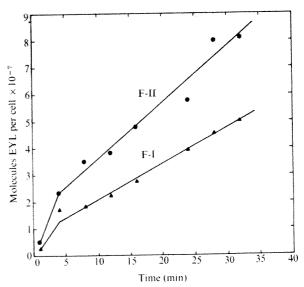


Fig. 1 Uptake data obtained for V79 cells incubated with Fig. 1 Uptake data obtained for V79 cells incubated with egg yolk lecithin (EYL) vesicles at 25° C. Vesicles were prepared by ultrasonic irradiation of radiolabelled EYL in Gey's balanced salt solution¹¹¹. The radiolabelled lipid was prepared by adding a trace amount of ∼5 Ci mmol⁻¹ ³H-distearoyl lecithin to EYL to give a product with a final specific activity of 1 mCi mmol⁻¹ EYL. The lipid dispersion was fractionated by get permeation chromatography³ into was fractionated by gel permeation chromatography<sup>3</sup> into multilamellar (F-I) and unilamellar (F-II) fractions, and diluted in each case to a concentration of 0.09 mM lipid phosphorus. In a typical experiment, a culture plate containing  $\sim 10^7$  cells was washed in Gey's, followed by the addition of the labelled vesicle suspension. After incubation the plate was washed thoroughly, the cells briefly trypsinised, and the resulting cell suspension repeatedly centrifuged and resuspended in Gey's. The radioactivity in the final pellet was determined, and the total number of phospholipid molecules taken up per cell was calculated. Error is ±5%.

The uptake of phospholipid from either unilamellar or multilamellar vesicles by cells was insensitive to inhibitors of energy metabolism (30 mM NaN3; 0.1 mM dinitrophenol). Cells pretreated with 2% glutaraldehyde (2 h at 4° C) before uptake showed about a 25% reduction in the amount of phospholipid taken up after 1 h of incubation. These observations strongly suggest that the incorporation of exogenous phospholipid from vesicles by the cultured cell system examined here is not energy dependent. Accordingly, in considering the possible mechanisms of uptake, we rule out active processes such as endocytosis, and consider only the following: (i) vesicle-cell fusion; (ii) phospholipid exchange, similar to that described for in vitro 13,14 systems in which phospholipid molecules from the vesicle suspension and plasma membrane of the cell are interchanged and (iii) adsorption, in which the exogenous

Table 1 Uptake by Chinese hamster V79 cells of <sup>14</sup>C-dioleyl lecithin vesicles containing 3H-inulin\*

And the second s	c.p.m. found treated	in vesicle- cells†	Ratio (14) 3H c.	C c.p.m./ p,m.)
	14C-dioleyl lecithin	<sup>3</sup> H-inulin	Observed	Expected
Multilamellar vesicles 2° C 37° C	126 370	~5 108	≥ 25 3.4	0.41 0.41
Unilamellar vesicles 2° C 37° C	220 800	42 1,000	5.2 0.8	0.44 0.44

<sup>\*</sup> Doubly labelled vesicles were prepared by cosonication of <sup>14</sup>C-dioleyl lecithin and <sup>3</sup>H-inulin. The vesicle fractions containing the trapped marker were subsequently separated from free inulin by chromatography on Sepharose 4B. No leakage of the trapped marker could be detected during this experiment. † Protocol as in Fig. 1; 1 h incubation.

phospholipid from the applied vesicles is adsorbed to the cell surface, resulting in a net transfer of lipid to the cell.

If fusion is involved in the interaction of phospholipid vesicles with cultured cells, then the aqueous contents of such vesicles should be released into the cytoplasm of vesicle-treated cells after each fusion event. To test this possibility, vesicles comprised of 14C-dioleyl lecithin and containing 3H-inulin as a marker for the internal aqueous volume were used in an uptake experiment (Table 1). If all the phospholipid enters the cell by fusion, the ratio of phospholipid to inulin radioactivity found associated with vesicle-treated cells should be that of the applied vesicle suspension. Table 1 shows the amounts of <sup>14</sup>C-dioleyl lecithin and 3H-inulin found associated with cells treated with either multilamellar or unilamellar vesicles together with the observed and expected ratios of c.p.m. of "C to tritium. The observed ratios were calculated from the 14C-lecithin and 3H-inulin taken up by the cells for each treatment, whereas the expected values were derived directly from the measured ratios of the applied vesicle fractions. In each case the amount of the trapped marker, 3H-inulin, which became cell-associated was not proportional to the

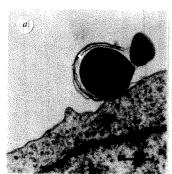




Fig. 2 Transmission electron micrographs of V79 cells incubated for 1 h at 37°C (a) with multilamellar vesicles generated from EYL, and (b) with unilamellar vesicles generated from dioleyl lecithin. In (b), cells were pretreated with cationised ferritin (see text). Cells were pre-fixed in 0.1 M cacodylate buffer (pH 7.2) containing 2% glutaraldehyde, fixed in 1%  $OsO_4$ , stained in uranyl acetate and embedded in Epon. Thin sections were stained with lead citrate. The bar represents 0.5  $\mu$ m.

amount of phospholipid taken up. For example, for multilamellar vesicles at 37° C, the observed ratio of phospholipid c.p.m. to inulin c.p.m. was 3.4, compared with an expected value of 0.41. If the trapped marker can become associated with the cell only as the result of fusion, we calculate that at 2° C, only small amounts of the observed phospholipid uptake (<2% for multilamellar and  $\sim10\%$  for unilamellar vesicles) can be accounted for by fusion, whereas at 37°C, more significant fractions of the lipid uptake ( $\sim 10\%$  for multilamellar and  $\sim 50\%$  for unilamellar vesicles) could enter the cells by such a mechanism. The remaining lipid would have to be taken up by some other pathway such as phospholipid exchange or adsorption. Experiments are in progress to test these possibilities. Similar studies of cells pretreated with glutaraldehyde demonstrated that the association of the trapped marker with vesicle-treated cells was not significantly inhibited, thus confirming the supposition that vesicle uptake is not an active process.

To demonstrate unequivocally that a fusion process is involved in the overall mechanism of uptake of unilamellar vesicles by cultured cells, we require in addition to the uptake of phospholipid and trapped marker, that the following criteria be met. First, the trapped marker must be truly soluble in the internal aqueous space of the vesicle.

The use of lipid vesicles bearing a net charge to entrain soluble markers of opposite charge raises the question of whether such markers are bound or associated with the wall of the lipid vesicle. If such an interaction exists, the bound marker might enter the cell by some internalisation mechanism and not by fusion. Second, the marker once associated with the cell must be demonstrated to be free within the cytoplasm of the cell and not compactmentalised either in the lysosomal apparatus or in some other membrane-bound vesicular body. Although we believe that the first condition has been fulfilled in our studies, we have no sufficient proof that the second criterion has been met. Thus we can only suggest vesicle-cell fusion as an explanation for our observations. Nevertheless, these studies should provide a useful basis for achieving modification of the surface properties of mammalian cells.

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# Yeast mitochondrial RNA does not contain poly(A)

POLY(A) is a universal constituent of eukaryotic messenger RNAs with the exception of histone mRNA1. Recently Penman and Attardi<sup>3</sup> have demonstrated that poly(A) was also present in mitochondrial RNA from animal cells. This finding was o interest to us for two reasons. First, it suggested a simple means of isolating mitochondrial mRNAs and, second, I suggested that the similarity between prokaryotes and mitochondria might not be as close as previously suggested. We therefore looked for poly(A)-containing RNA (poly(A)-RNA in the simple eukaryote Saccharomyces carlsbergensis.

Intact yeast mitochondria are difficult to isolate quickly from whole cells. Since rapid isolation should be a prerequisite for the characterisation of RNA pulse labelled for short periods, we preferred to pulse label isolated protoplasts. Using this system McLaughlin *et al.*<sup>5</sup> have detected considerable amounts of poly(A)-RNA in yeast polysomal RNA. Details of our protoplast preparation are described elsewhere (G.S.P.G., and R. O. Poyton, unpublished).

Yeast protoplasts were pulse labelled for 30 min with  $^3$ H-adenine, then quickly cooled and lysed by osmotic shock. After the removal of nuclei and cell debris, mitochondrial and post-mitochondrial fractions were prepared and RNA extracted from these by the hot-phenol-chloroform method of Penman? After extraction the RNA was precipitated with two volumes of ethanol and stored at  $-20^{\circ}$  C overnight. The recoveries of both  $^3$ H-labelled RNA and added  $^3$ P-labelled poly(A), as determined by acid-insoluble radioactivity, were always greater than  $90^{\circ}$ 0.

To test whether poly(A)-RNA was present, aliquots fapproximately 20,000 c.p.m.) of the mitochondrial and cell-sap RNA were fractionated on oligo(dT) cellulose columns as described by Faust et al.8. The results of such a fractionation are given in Fig. 1. It can be seen that, whereas 30% of the cell-sap RNA is retained by the column and elutes at low salt concentrations (Fig. 1b), less than 0.6% of such material is present in the mitochondrial RNA (Fig. 1a). The binding of the mitochondrial RNA was unaffected by previous denaturation (2 min, 100° C in 0.5 mM EDTA, pH 7.5). As an internal control 32P-labelled poly(A) was added and indeed eluted exclusively at low salt concentrations. To exclude the possibility that the cell-sap poly(A)-RNA was bound by a nonspecific process, competing poly(U) was added to a second aliquot of this RNA plus internal 32P-labelled poly(A). As expected, both the 3H and 32P radioactivity was no longer retained by the oligo(dT) cellulose (Fig. 1c), demonstrating that the cellsap RNA was indeed bound by its poly(A) moiety. Since the recovery from our oligo(dT) cellulose columns was 95-110%, we conclude that mitochondrial RNA pulse-labelled for 30 min. does not contain a poly(A) sequence of sufficient length to bind it to the oligo(dT) cellulose column. Identical results were obtained using chromatography on poly(U) Sepharose

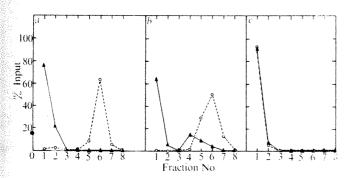
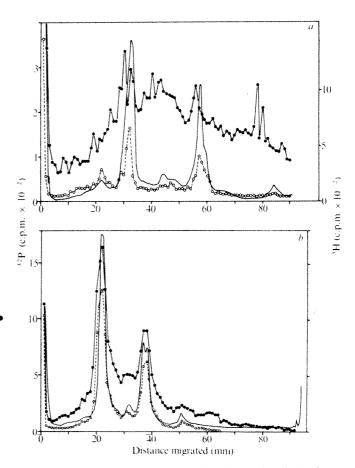


Fig. 1 Fractionation of pulse-labelled RNA on oligo(dT) cellulose columns. 4 g of yeast (S. carlsbergensis NCYC 74) protoplasts were labelled for 30 min at 28° C with <sup>3</sup>H-adenine (17 mCi l<sup>-1</sup>) in 200 ml growth medium, supplemented with 1.0 M sorbitol; cell-sap and mitochondrial RNA was prepared as described in the text; aliquots (20,000 c.p.m.) in 0.5 M KCl, 0.01 M Tris, pH 7.5, were mixed with <sup>32</sup>-P-labelled poly(A) (10,000 c.p.m.), prepared as described<sup>14</sup>, and applied to the oligo(dT) cellulose column. The flow-through material was passed again over the column. Fraction 1: final flow-through; fraction 2, elution with 0.5 M KCl, 0.01 M Tris; fraction 3, 0.25 M KCl, 0.01 M Tris; fraction 4, 0.1 M KCl, 0.01 M Tris; fraction 7, 95% formamide, 0.01 M Tris; fraction 8, 0.1 M NaOH. The fractions were then precipitated with 5% trichloroacetic acid after the addition of 100 μg bovine serum albumin. The precipitates were collected on Whatman glass fibre filters (GF-81) and counted. Δ, 30 min <sup>3</sup>H-adenine pulse-labelled RNA; 0---0, <sup>32</sup>P-poly(A). a, Mitochondrial RNA; b, cell-sap RNA; c, cell-sap RNA + poly(U).



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Fig. 2 Polyacrylamide gel analysis of pulse-labelled mitochondrial RNA from yeast. Electrophoresis conditions: 2.4% acrylamide gels using the buffer system of Peacock and Dingman<sup>15</sup>. After scanning at 260 nm (——), gels were frozen at -70° C, sliced, extracted and counted<sup>16</sup>. C, <sup>3</sup>H-adenine longlabelled mitochondrial RNA added as internal marker during electrophoresis; •, <sup>32</sup>P pulse profile. a, 4 min pulse; b, 15 min pulse.

To minimise the chance that poly(A)-RNA was missed in our experiments because of degradation during the isolation of the mitochondria, the cell-sap fraction was maintained in ice for the same length of time used for the mitochondrial purification (2 h). The finding of 30% of the cell-sap RNA as poly(A)-RNA renders this possibility unlikely.

We considered it possible that the in vivo turnover rate of poly(A)-mitochondrial RNA was so rapid that very little could be detected in a 30-min pulse in contrast to the cell-sap system. We therefore prepared mitochondrial RNA pulse-labelled for 4, 15 and 30 min, respectively. Polyacrylamide gel electrophoresis of two of these preparations is shown in Fig. 2. The 4 min pulse-labelled preparation clearly contains much heterogeneous RNA, indicating that processing and maturation of at least rRNA, has not occurred. This is clearly evident in the 15-min pulse-labelled material. The presence of intact endogenous rRNAs in these preparations, however, excludes trivial explanations for the heterogeneous RNA such as degradation. The 4 min and 30 min pulse preparations were also applied to the oligo(dT) cellulose column (Table 1). Again less than 0.5% of the 32P-labelled RNA was retained by the column in contrast to the complete retention of the internal 3H-labelled poly(A) control. Total RNA from protoplasts pulse-labelled for 5 min with 3H-adenine contained 20% poly(A)-RNA. Moreover, we were able to show considerable amounts of poly(A)-RNA in the mitochondrial RNA from chick fibroblasts (Table 1). Finally, we have analysed RNA synthesised by isolated yeast mitochondria in vitro for poly(A) content. Again less than 0.4% poly(A)-RNA could be detected.

As explanation of these results, two possibilities remain: either there is no poly(A)-mitochondrial RNA in yeast or the

Table 1 % Poly(A)-RNA in pulse-labelled mitochondrial RNA

Labelling condition	Organism	Radio- active pre- cursor	Pulse time (min)	Input applied to oligo(dT) cellulose (c.p.m.)	RNA re- tained
In vivo	Yeast	32 <b>P-P</b> i	4	10,500	0.5
In vivo	Yeast	32P-P <sub>1</sub>	30	42,000	0.5
In vivo	Yeast	<sup>3</sup> H-adenine	30	4,840	0.6
In vitro	Yeast	<sup>3</sup> H-UTP	20	5,100	0.4
In vivo	Chick	<sup>3</sup> H-uridine	15	16,100	34.4

Yeast protoplasts (5–15 g, 20 g  $l^{-1}$ ) were pulse-labelled with either <sup>32</sup>P-orthophosphate (25–40 mCi  $l^{-1}$ ) or <sup>3</sup>H-adenine (4 mCi  $l^{-1}$ ) and mitochondrial RNA isolated as described in Fig. 1. To measure in vitro RNA synthesis, isolated mitochondria were incubated as described<sup>11</sup>, except that the medium was supplemented with 50 µM <sup>3</sup>H-UTP (specific activity 10<sup>6</sup> c.p.m. mmol<sup>-1</sup>). Chick fibroblasts were grown and pulse-labelled with <sup>3</sup>H-uridine as described<sup>12</sup> and mitochondria were purified as described by Borst et al.13. RNA was prepared and oligo(dT) cellulose chromatography performed as described in Fig. 1. The % retained is defined as the counts eluting in fractions 4-8.

poly(A) region is so short that it cannot be retained by the oligo(dT) cellulose and poly(U) Sepharose columns. Preliminary experiments showed that while polyacrylamide gel electrophoresis was capable of resolving a 50 nucleotide poly(A) segment<sup>5</sup> derived from cell-sap RNA by ribonuclease digestion9, no such component was present in mitochondrial RNA. Under these conditions, however, a poly(A) tract of less than 30 nucleotides would not have been detectable above the high background of completely digested RNA peaking ahead of the bromophenol blue marker. Since Jacobson et al.9 showed that a poly(A) segment of 25 was sufficient to bind Dictyostelium nuclear RNA to poly(U) Sepharose, we presume that if mitochondrial RNA contains a poly(A) segment, it must be considerably shorter than this. Such a poly(A) region differs so much from its homologue in the animal cell, that its possible existence becomes somewhat a semantic problem.

To us the most likely interpretation of our data is that mitochondria from yeast contain no poly(A)-RNA in contrast to animal mitochondria and in common with the RNA of prokaryotes and histone mRNA. Such a result is consistent with a prokaryotic origin for mitochondria and tempts us to suggest that poly(A)-mRNA in animal mitochondria is the result of adaption of a 'prokaryotic' genetic system to the demands of life in the higher eukaryote which occurred parallel to the loss of genetic information in the animal mitochondrial DNA (the potential coding capacity of yeast mitochondrial DNA is two to five times that of animals).

Finally, our results are in conflict with those of Cooper and Avers10, who reported poly(A)-mitochondrial RNA in yeast mitochondria. We attribute their results to contamination of the mitochondrial preparations with intact protoplasts.

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### Large plasmid in Agrobacterium tumefaciens essential for crown gall-inducing ability

THE gram-negative bacterium Agrobacterium tumefaciens induces crown gall tumours in many, mostly dicotyledonous. plants. Zaenen et al.1 demonstrated the presence of one or more large plasmids in a number of crown gall-inducing Agrobacterium strains belonging to seven different Agrobacterium groups. They were not able to find such plasmids in eight non-pathogenic Agrobacterium strains belonging to four of the same groups<sup>2,3</sup>. They therefore formulated the hypothesis that the genetic information for the tumour-inducing principles in crown gall-inducing Agrobacterium strains is carried by one or several large plasmids.

Here we determine whether the large plasmids present in crown gall-inducing Agrobacteria are essential to the tumourinducing capacity of such strains. We show that, in a nontumorigenic derivative of a crown gall-inducing strain, the stable conversion to non-tumorigenicity is correlated with a concomitant loss of the large plasmid present in the parental strain. Second, we show for one tumour-inducing Agrobacterium strain that curing this strain of its plasmid in all cases resulted in the stable conversion of the cured strains to nontumorigenicity, the correlation between the loss of the plasmid and the loss of the tumour-inducing plasmid (TIP) being 100%

The crown gall-inducing Agrobacterium tumefaciens strain IIB and two of its derivatives, the tumorigenic strain IIBV7 and the non-tumorigenic strain IIBNV6, both isolated from strain IIB as single colonies, were compared (Braun, A. C., unpublished). Using ultracentrifugation on neutral sucrose gradients and caesium chloride-ethidium bromide equilibrium density centrifugation in the conditions described by Zaenen et al.1, we demonstrated the presence of a large plasmid in A. tumefaciens strains IIB and IIBV7. Fractions containing the plasmid peaks were examined under the electron microscope and large double-stranded DNA supercoiled molecules were photographed. Only the non-tumorigenic strain IIBNV6 did not contain a large plasmid when examined repeatedly.

Hamilton et al.5 reported that the crown gall-inducing strain C58 can very reproducibly be converted to a non-tumorigenic

Table 1	Bacterial colonies	which can induce crown	i gall tumours also ha	rbour large plasmids

No. generations of growth at 37° C  0 5 5 5 5		Table 1 Bacter	rial colonies which o	an induce crown gall	tumours also harbo	ur large plasmids	
0 5 5 5 — — — — — — — — — — — — — — — —	 of growth at	No. colonies tested for pathogenicity	forming colonies	tested for the pre-	tive colonies	tested for the pre-	No. plasmid-nega- tive colonies
2 5 5 5 — — — — — — — — — — — — — — — —	3,0	5	5	-	agence of the c		MATERIA
10	ž	5	5	***************************************	manufacture.	ar manusis	_ SPECIALISM
10	ā	5	5	2	2		2
19 15 3 3 3	10	<b>P</b> O	8	8	8	2	2
	19	15	3	3	3	8	8
38 15 0 — 1	38	15	0	population	vanninkapi.	1	

strain by simple growth at 37° C. The resulting non-tumourinducing bacteria do not revert to tumorigenicity. We found that derivatives of A. tumefaciens strain B6 could neither be cured of their plasmid nor converted to non-tumorigenicity by growth at 37° C. For further study we therefore decided to concentrate on strain C58. By neutral sucrose gradient and caesium chloride-ethidium bromide centrifugation and subsequent electron microscopy, strain C58 was shown to harbour a large plasmid. Length contour measurements were carried out on open circular molecules. Analysis of the results showed an unimodal distribution, the mean contour length being  $61.8\,\pm\,1.1~\mu m$  (s.d.).

To obtain independently-isolated non-tumorigenic derivatives and to check the reproducibility of the conversion to non-tumorigenicity, strain C58 was streaked out for single colonies on a YEB agar plate1 and incubated for 5 d at 37° C. Six of the resulting colonies were separately collected, diluted and plated for single colonies on six different YEB agar plates at 28° C. Twenty-five colonies from each plate were tested for tumour induction on wounded seeds of Pisum sativum<sup>6</sup>. All 150 colonies tested proved to be non-tumorigenic. A total of 12 non-tumorigenic colonies (two from each plate) were cultured at 28° C and their DNA analysed on neutral sucrose gradients1. These non-tumorigenic bacteria were found to have lost the large plasmid present in the parental strain C58.

These results provide strong support for the hypothesis that the genetic information for the tumour-inducing principle in crown gall-inducing Agrobacterium strains is carried by one or more plasmids. The loss of tumour-inducing ability and loss of the plasmid, however, may be two unrelated events both brought about by growth at 37° C. If this were the case one would expect that in conditions where neither the curing of the plasmid nor the conversion to non-tumorigenicity would have occurred for all the population, one would find some bacteria for which only one of these events would have taken place. In order to realise such conditions we studied the kinetics of appearance of non-tumorigenic and of plasmid free bacteria in a strain C58 culture grown at 37° C.

An exponential culture of strain C58 grown in YEB medium at 28° C was diluted in fresh YEB medium to about 104 cells ml-1 and incubated at 37° C. When a titre of 109 cells ml-1 was reached, the culture was diluted to a titre of 104 cells ml-1 into fresh medium and further incubated at 37° C. This procedure was repeated several times. At different intervals samples were taken, the bacteria were diluted and plated for single colonies on YEB plates at 28° C. From each sample, several single colonies were tested on pea seedlings for tumour

Several tumourigenic and non-tumourigenic colonies derived from different samples were tested for the presence of a large plasmid. To be able to handle more colonies when searching for the presence of plasmids in Agrobacteria we modified the neutral sucrose gradient technique of Zaenen et al.1 by adding I ug ml-1 ethidium bromide (final concentration) to our sucrose gradients. Plasmid bands can be seen when the nitrocellulose centrifuge tubes are illuminated with long wave ultraviolet light. This eliminates the time consuming radioactive labelling, fractionating and liquid scintillation counting procedures.

All bacterial colonies that could induce crown gall tumours also harboured the large plasmid (Table 1), whereas all bacteria which had lost the ability to induce tumours had also lost the

plasmid. In all experiments special care was taken to make certain that we were not dealing with contaminants. This could easily be achieved by the use of a set of virulent Agrobacterium phages towards which strain C58 shows a characteristic sensitivity pattern.

As in all our experiments the correlation between loss of tumour-inducing capacity and loss of a large plasmid was found to be a 100%, we conclude that the presence of this plasmid is essential to the tumour-inducing ability of these strains. The most likely explanation for this would be that the plasmid in itself carries the genetic information for the tumour-inducing principle.

Such a conclusion, however, can only be reached if one can demonstrate induction of crown gall tumours with purified plasmid DNA. Our observations point to the possibility that bacterial plasmids could be oncogenic. Further work will be required to decide whether the role of this plasmid in tumour induction is directly due to the fact that it carries the genes of the tumour-inducing principle or merely because it somehow promotes the transfer of the TIP from the bacterium to the plant cell.

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# Multiple divergent copies of endogenous C-type virogenes in mammalian cells

It is characteristic of endogenous C-type viruses that all individual animals of a species (as well as all tissues of each animal)

contain, in the chromosomal DNA, nucleic acid sequences that can code for the production of complete C-type viral particles. It is postulated that these sequences (C-type virogenes) are transmitted from parent to progeny with the other cellular genes1. Nucleic acid hybridisation, using either single-stranded or double-stranded 3H-DNA transcripts or labelled 70S RNA as viral probes, has revealed C-type viral nucleic acid sequences in DNA from uninfected tissues of avian<sup>2-5</sup>, murine<sup>6,7</sup>, porcine<sup>8</sup>, feline<sup>9-12</sup> and Old World monkey species<sup>13</sup>.

We have now examined the reiteration frequency of mouse, rat, pig, cat and baboon viral nucleic acid sequences in tissues and cell cultures of the species of origin of these viruses and in exogenously infected cells of heterologous species. While DNA from each species contains multiple copies of endogenous virogene sequences per haploid cellular genome (5-15), cell lines of heterologous species infected with and producing high titres of these C-type viruses contain fewer copies (one or two per haploid genome). The thermal stability of DNA:DNA hybrids demonstrates that the endogenous virogenes are composed of families of genes with related but not identical nucleic acid sequences.

We examined a xenotropic (S-tropic) virus isolated from a murine BALB/3T3 cell line14; the RD114/CCC group of endogenous cat viruses<sup>15,18</sup>; a C-type virus isolated from normal baboon tissue17; a porcine C-type virus spontaneously released by the pig kidney cell line, PK(15)8; and a rat C-type virus produced by a rat thymus cell line18.

The reassociation kinetics shown in Fig. 1 can be used to estimate relative gene frequencies by determination of the half  $C_0t$  values (the midpoint of the renaturation curve)<sup>19</sup>. Figure 1a shows that the half  $C_0t$  value for the hybridisation of the murine C-type viral <sup>3</sup>H-DNA probe to normal BALB/c strain mouse liver or to BALB/3T3 cell line DNA is  $1.1 \times 10^2$ – $1.2 \times 10^2$  $mol \times sl^{-1}$ ; whereas the half  $C_0t$  value for hybridisation with the DNA of either a canine or rabbit cell line infected with this virus is 1.5 imes 103. The half  $C_0 t$  value for the self-annealing of unique sequence rabbit and dog (Fig. 1), as well as mouse, cat, baboon and human cellular DNA has varied between  $1 \times 10^3$ and  $2 \times 10^3$  mol  $\times$  s  $1^{-1}$  (ref. 13). There is, therefore, only one or at most two copies of mouse C-type virus in the heterologous infected dog or rabbit cell lines, and there are approximately ten times this number of copies in the mouse cell line or in normal mouse tissue.

Sequences homologous to probes prepared from the endogenous baboon virus can be found in the DNA of various tissues of normal baboons<sup>13</sup>; as shown in Fig. 1b, the half  $C_0t$ value for this hybridisation is  $1.0 \times 10^2$ – $1.4 \times 10^2$ . In contrast, a cloned cell line infected with the baboon virus contains only one copy per haploid cell DNA (half  $C_0t$  value is  $1.0 \times 10^3$ ).

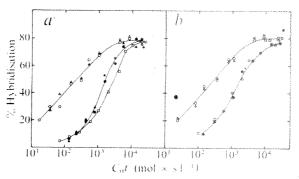


Fig. 1 Hybridisation of murine and baboon C-type viral H-DNA probes to DNA extracted from normal murine and baboon tissues and cell lines, and from cells from heterologous species infected with these viruses. The 3H-thymidine-labelled DNA probes were synthesised from detergent-disrupted C-type virus in the presence of actinomycin D<sup>27</sup>. The specific activity of the <sup>8</sup>H-DNA was 1.5 × 10<sup>7</sup> c.p.m. µg<sup>-1</sup>. The <sup>8</sup>H-DNA probes contained 50-75% of their respective 70S viral RNA sequences at a <sup>8</sup>H-DNA. <sup>28</sup>P-viral RNA molar ratio of 1.513, indicating that they contained most of the sequences present in viral RNA and that these sequences were in proportions similar to their content in 70S RNA. Cellular DNA was extracted from tissues and cell lines18. All DNAs were treated sonically to yield a mean size of 6-88 (the size of the <sup>3</sup>H-DNA probes) as determined by centrifugation on alkaline sucrose gradients<sup>13</sup>. DNA:DNA hybridisations were incubated at 65° C in reaction mixtures containing 0.01 M Tris, pH 7.4, 0.75 M NaCl, 2  $\times$  10<sup>-3</sup> M EDTA, 0.05% Sodium dodecyl sulphate, 10,000–20,000 c.p.m. ml<sup>-1</sup> of <sup>3</sup>H-DNA and 1–3 mg of cellular DNA per ml. Hybridisations were started by heating the mixtures to 98° C for 10 min, cooling on ice to 4° C, and incubating at 65° C. At various times (from 15 min to 96 h) 0.05 ml portions were removed and frozen at -80° C until digested with the single-strand-specific nuclease, S1, as described13. Cal values  $(C_0)$  is the concentration of cellular DNA in moles of nucleotide per litre, and t is the time in seconds) were calculated as suggested by Britten and Kohne<sup>28</sup> as A<sub>260</sub> per ml per 2 × h, and corrected to a monovalent cation concentration of 0.18 M<sup>28</sup>, a, Annealing of <sup>3</sup>H-DNA probe prepared from the xenotropic (S-tropic) BALB/c murine C-type virus<sup>14</sup> grown in canine thymus cells (FCf2Th) to DNA extracted from the BALB/3T3 cell line ( normal BALB/c liver (A), the canine thymus cell line (FCf2Th) infected with the S-tropic virus (♠); and a rabbit corneal cell line (SIRC)<sup>14</sup> infected with this virus (♠). Various concentrations of unique sequence rabbit cell line (SIRC) DNA (the highly reiterated DNA sequences that anneal by a  $C_0$ / of 50 were removed by fractionation on hydroxylapatite) and 4  $\times$  10 acid precipitable c.p.m. of 3H-thymidine-labelled unique sequence SIRC DNA (0.5 µg) were annealed (☐) as described above. b. Annealing of <sup>3</sup>H-DNA probe prepared from the baboon virus<sup>12</sup> grown in the human rhabdomyosarcoma (A204) cell line to DNA extracted from liver  $(\triangle)$  and lung  $(\bigcirc)$  tissue of two normal baboons, and from the canine thymus cell line (FCf2Th) infected with baboon virus ( ). Self-annealing of the canine cell line (FC(2Th) unique sequence DNA (

Table 1 Nucleic acid reassociation kinetics of viral 3H-DNA probes to the DNA of various tissues, cell lines, and cells infected with visus

Virus*	Tissue	$C_0 t_{1/2} \dagger$	Cell line‡	$C_0 t_{1/2}$	Cell line	C of 1/2
Murine (S-tropic)	Mouse liver	$1.2\times10^{2}$	BALB/3T3 S2CL3	$1.1 \times 10^{2} \\ 1.2 \times 10^{2}$	(infected) S-tropic/SIRC S-tropic/FCf2Th	1.5 × 10 <sup>3</sup> 1.2 × 10 <sup>3</sup>
Baboon (M7)	Baboon lung liver spleen	$1.0 \times 10^{2}$ $1.4 \times 10^{2}$ $1.4 \times 10^{2}$	NT	**************************************	M7/FCf2ThCL5	1.0 × 10 <sup>s</sup>
Feline (RD114)	Cat liver kidney	$1.8 \times 10^{2}$ $1.8 \times 10^{2}$	CCC CL6 FFc60WF	$\begin{array}{c} 2.0 \times 10^{2} \\ 1.9 \times 10^{2} \end{array}$	CCC/FCf2Th RD114/M413	$1.5 \times 10^{3}$ $2.0 \times 10^{3}$
Rat (RT21C)	Rat liver	$0.6 \times 10^2$	RT21C LLC-WRC256	$1.2 \times 10^{2}$ $0.9 \times 10^{2}$	NT§	2.0 / 10
Porcine (PK15)	Pig liver	$1.5\times10^{2}$	PK(15)	$1.5 \times 10^2$	NT§	

NT, not tested.

<sup>3</sup>H-DNA probes were prepared from the viruses as described in Fig. 1. S-tropic murine virus<sup>14</sup> was grown in the canine thymus (FCf2Th cell line. Baboon C-type virus (M7)<sup>17</sup> was grown in the human rhabdomyosarcoma cell line (A204), and RD114 in RD cells<sup>13</sup>. Rat (R721C)<sup>14</sup> and pig (PK(15))<sup>8</sup> viruses were spontaneously released from their respective cell lines.

† Half C<sub>0</sub>t values represent the midpoint of the reannealing curves<sup>19</sup>. The reannealing rate of dog, baboon, human, cat, rabbit and mouse unique sequence cellular DNA has been shown<sup>13</sup> to range between a half C<sub>0</sub>t of 1.0 × 10<sup>3</sup>-2.0 × 10<sup>3</sup>.

‡ The cells are described in the legends to Figs 1 and 2. S2CL3 is a BALB/c cell line spontaneously producing mouse C-type virus<sup>32</sup>, and LIC-WRC256 is a rate carcinoma line producing rate C-type virus<sup>32</sup>.

LLC-WRC256 is a rat carcinoma line producing rat C-type virus<sup>32</sup>

§ These viruses do not replicate to any extent in any mammalian cell line tested.

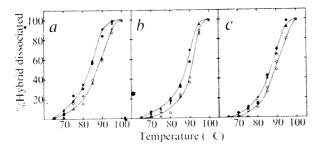


Fig. 2 Thermal stability of hybrids formed between viral <sup>3</sup>H-DNA probes and cellular DNA of various tissues and virus-infected cell lines. After hybridisation to a  $C_0t$  of  $10^4$ , aliquots (containing 600-1,200 c.p.m.) were heated for 5 min in 0.75 M NaCl at the indicated temperatures. The amount of hybrid remaining was determined with the single-strand-specific nuclease, S<sub>1</sub>. a, Thermal stability of hybrids formed between a <sup>3</sup>H-DNA probe prepared from the xenotropic (S-tropic) BALB/c virus<sup>14</sup> grown in a canine thymus cell line (FCf2Th) and DNA extracted from the S-tropic virus-infected canine cell line (O); the virusinfected rabbit cell line (SIRC)¹¹ (△); mouse liver (♠); and a BALB/3T3 cell line (♠). b, The ³H-DNA probe prepared from RD114 virus grown in RD cells¹⁵ was hybridised with DNA extracted from a canine thymus cell line infected with the endogenous cat virus, CCC (Ο); from a line of human embryonic fibroblasts (M413) infected with RD114 (Δ); from domestic cat liver ( ); and from either CCC CL6 or FFc60WF feline cell lines 31 (A). c, The 3H-DNA probe prepared from an endogenous baboon virus (M7) grown in the human rhabdomyosarcoma line A204<sup>17</sup> was hybridised with DNA extracted from the canine thymus cell line infected with M7 (○); from baboon liver (●); and baboon lung (A).

Table 1 summarises the half  $C_0t$  values obtained with  ${}^3H$ -DNA probes prepared from several mammalian C-type viruses. A human or canine cell line infected with the endogenous domestic cat virus, RD114, contains only one copy in the cell DNA (half  $C_0 t$  value is  $1.5 \times 10^3$  to  $2.0 \times 10^3$ ), whereas normal feline tissues and cell lines contain multiple copies of genes related to the information in this virus (the half Cot values range from  $1.8 \times 10^2$  to  $2.0 \times 10^2$ ). <sup>3</sup>H-DNA probes prepared from a porcine virus (PK(15)) and a rat virus (RT21C) were also hybridised, respectively, to the DNA of normal pig and rat. Again, multiple copies of these viral gene sequences are present in the DNA of their species of origin (half Cot values are  $0.6 \times 10^2$  to  $1.5 \times 10^2$ ). Since neither of these viruses can replicate in the mammalian cell lines so far tested, we cannot determine the reiteration frequency of the viral genes in exogenously infected cells. Each of the five mammalian species examined thus contains multiple copies of their endogenous virogenes. In striking contrast is the situation where a cell is infected with and actively producing a heterologous C-type virus; only one to two copies of the virus information is present in the cellular DNA.

Labelled DNA probes prepared from mouse leukaemia viruses (Kirsten and AKR) grown in mouse cells have also revealed multiple copies (five to seven) in mouse cell DNA6.7, as described here using probes from the xenotropic BALB/c virus grown in heterologous cells. Furthermore, the mouse mammary tumour viruses, B-type viruses, are also present in multiple copies in normal murine cell DNA<sup>20</sup>. The results described here for the five mammalian systems may not apply to the subgroup E endogenous chicken virus, where fewer viral copies (one or two) have been reported in normal tissues<sup>5</sup>. Some mammalian cells transformed by avian sarcoma virus, however, acquire one viral copy per haploid genome4, as do the cells infected with heterologous mammalian C-type viruses.

To examine whether the multiple copies of C-type viruses in the cellular DNAs of these mammalian species represented identical copies of one set of virogenes or a family of related sets of virogenes, the thermal stability of the hybrids was determined. Base-pair mismatching results in the formation of hybrids that melt at a lower temperature—the effect of mismatched base pairs on the thermal stability is between 0.7° C and 1.6° C per 1% altered base pairs19. Figure 2 shows the melting curves obtained when single-stranded <sup>3</sup>H-DNA transcripts of murine, RD114, and baboon C-type viral RNA were hybridised to the DNA from normal murine, cat and baboon tissues, respectively, and to the DNA of heterologous mammalian cells infected with these viruses. As Fig. 2a-c shows, the hybrid formed with DNA extracted from the species of origin of each virus melted 2-4° C lower than the hybrid formed with the DNA of a heterologous infected cell line. This result did not depend on the cell line in which the virus was grown; for example, the thermal stability data obtained with DNA extracted from either the canine or human cell lines infected with the endogenous cat viruses (RD114/CCC group) was higher than that obtained with various normal cat cellular DNAs. Thus, the multiple copies of C-type viruses in these mammalian species (murine, feline and baboon) were not all identical in sequence to the endogenous viruses isolated from these species.

What is the evidence that the various endogenous C-type viruses isolated from each of these species are different? In the murine system, three endogenous C-type viruses have been isolated from the BALB/3T3 cell line that differ not only in their host range but also in some of their nucleic acid sequences14,21. The C-type viruses from two rat cell lines (LLC-WRC256 and RT21C) share only a partial (40-60%) nucleic acid sequence homology (unpublished observations). We suggest, therefore, that the other mammalian species tested will also contain sets of virogenes which will be only partially related to the ones that have so far given rise to infectious C-type viruses.

The presence of multiple copies of viral information is a useful criterion with which to identify the species of origin of an endogenous mammalian C-type virus. A feline virus (RD114) satisfies this criterion, as do the mouse, baboon, rat and pig C-type viruses. Moreover, it is striking that the copy number clusters in a relatively narrow range (5 to 15 copies per haploid genome). Cells (including clonal isolates) and fresh tissues, to the extent that they have been examined, have comparable numbers of copies. These multiple copies of genetically transmitted C-type virus information may be present in the germ cells of each animal of a species (virogene hypothesis1) or they may evolve by recombination and gene duplication during the lifetime of the animal (protovirus hypothesis22). Taken together with experiments showing considerable expression both of viral RNA and viral specific proteins in embryos and various normal tissues<sup>23-26</sup>, the results described here suggest that C-type genes have been preserved selectively during evolution and that their expression has some normal physiological function. Whether during their evolution as cellular genes the virogenes of a single species have diverged from one another in function, as they have in structure, remains to be determined.

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# Scanning immunoelectron microscopy of mouse B and T lymphocytes

ALTHOUGH B and T lymphocytes (derived from the bone marrow or thymus, respectively) are functionally different, they are not morphologically distinguishable by ordinary light and transmission electron microscopy (TEM). Thus immunospecific labels had to be developed for these methods to identify populations of lymphocytes1,2. Scanning electron microscopy (SEM) has been reported to show that T cells are smooth and B cells are villous3.4. This conclusion, however, is controversial, and it is uncertain whether T and B lymphocytes can be distinguished by SEM appearance5.6. We have now used immunospecific latex markers, which can be visualised by SEM, to identify cells with known T and B-associated antigenic surface determinants in the mouse. Mouse B cells have small stubby projections and are generally smoother than mouse T cells, which may be either villous or have few projections, but there is no distinct morphological difference.

An indirect latex labelling method, adapted from that of LoBuglio et al.7, was used to localise B-cell-associated and T-cell-dependent antigenic determinants on mouse lymphoid cells. The cells were treated with rabbit antisera to B or T-cellassociated antigens and then labelled with latex spheres coated with sheep anti-rabbit IgG (SARG). Rabbit anti-mouse Ig (B-cell-associated antigen, RAMG) was prepared by immunising rabbits with y-globulins precipitated twice with ammoniun sulphate and purified on DEAE. Rabbit anti-mouse brain (T-cell-associated antigen, RAMB) antiserum was prepared by immunising a rabbit with one brain equivalent in complete Freund's adjuvant (CFA) (Difco) in several subcutaneous sites boosting after I month and collecting the serum after a furthe month. The antiserum was heat-inactivated at 56° C for 45 min and absorbed with one-third volume packed mouse red blood

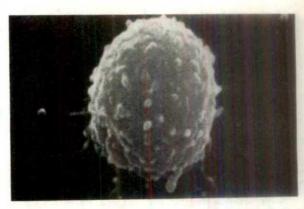


Fig. 1 Nu/Nu lymphocytes (B cells) sensitised with RAMB (T-cell-associated antigen). Cells were 5-7 µm in diameter and relatively homogeneous with a smooth surface. Usually 10-20 basal pseudopodial attachments and 50-100 micro-stubs covering the remaining surface could be visualised after attachment to glass. If the cells were treated with RAMB and followed by SARG-latex (see text), no latex label was visualised on the cell surfaces.

cells. Functionally, this antiserum plus complement remove all(1) helper cell activity for primary and secondary immun responses in mice, (2) concanavalin A and phytohaemaggluti mitogenic activity in mouse spleen populations and (3) cytotox effector cells, but had no detectable effect on plaque-formin cells, lipopolysaccharide mitogenic activity and precursor c memory B-cell populations as assessed by in vitro immur induction. SARG was prepared by immunising each of tw sheep with purified H chains of rabbit IgG in CFA intramu cularly. Booster injections were given and the sheep were ble 3 and 7 weeks later. The antisera were specific for IgG b immunoelectrophoresis and double diffusion in agar. Late particles nonspecifically adsorb y globulins, and latex sphere with adsorbed y globulin which has specific antibody activit will attach to the specific surface antigen7. One milligramme (



Nu/Nu lymphocytes sensitised with RAMG (B-cell associated antigen). Eighty per cent of cells treated with RAMB and SARG-latex (see text) show specific surface labelling for surface immunoglobulin (arrows). Usually a heavy background of latex particles (on the glass) accompanied positive labelling due to possible shearing and removal of surface label during preparation for SEM.

latex spheres (0.2 µm diameter) (Monsanto) was coated with 75 µl of SARG (SARG-latex) for 30 min at room temperature before labelling.

Mouse lymphocytes were prepared from the mesenteric lymph nodes of either congentially athymic, Nu/Nu, or BDFI mice. The lymph nodes were teased apart, cell aggregates were allowed to settle and then the cell suspensions were washed three times in basal salt solution. Functionally, Nu/Nu cells are primarily a B cell population<sup>8,9</sup>, and BDFI lymph node cells are a mixture of B and T cells; enriched T-cell preparations were obtained from BDFI lymph nodes by collecting the first 10 ml effluent fraction from a nylon wool column<sup>10</sup>.

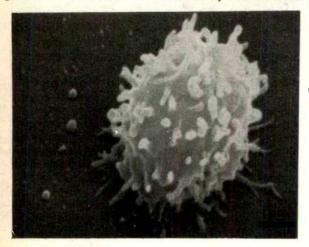


Fig. 3 BDF1 T cells sensitised with RAMG (B-cell-associated antigen). BDF1 lymphocytes (T cells) passed through the column showed heterogeneity in surface topographical characteristics. Usually 70–80% of the cells (5–7 μm in diameter) were villous, with 20–100 surface projections (50–800 nm long). The remaining 20–30% of the cells had smoother membranes, some without microvilli (not shown). If the cells were treated with RAMG and SARG-latex (see text), no surface labelling was seen.

Cell suspensions were prepared for latex labelling by settling 1-2×106 cells on to a 2 cm2 circular glass coverslip for 30 min at 37° C. The cells were fixed to the glass in 1% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, for 30 min and rinsed twice in buffer. Sensitising or coating antiserum (RAMG or RAMB) was added for 30 min at room temperature and unreacted antiserum was rinsed off with PBS. Before addition of SARGlatex label, coverslips were rinsed in 0.2 M diphosphate, pH 9.2. SARG-latex was added for 30 min and unattached particles were washed off by flushing with 0.2 M diphosphate. The remaining antibody-ligated latex particles attached to the cell surface were stabilised by a second fixation in glutaraldehyde. γ Globulins (SARG) adsorbed on the latex particles may dissociate from the latex at low molarities and pH between 5.5 and 9.0, so that postfixation is needed to prevent dissociation of the latex from the cell surface. Even so, during SEM preparation the shearing forces on the large latex spheres may remove them from the cell surface, and we usually found many shed latex spheres with positive labelled cells. Labelled cells were dehydrated in a graded series of ethanol concentrations. The specimens were continued through Freon 113 and Freon 13 for critical point drying11, coated with 400 Å of palladium and examined on an ETEC U-1 scanning electron microscope with an accelerating voltage of 30 kV and specimen tilt of 30° or 45°.

SEM of labelled B lymphocytes (Nu/Nu cells) showed that they had a relatively smooth surface membrane, with prominent, stub-like digitations, but no distinct finger-like microvilli Figs 1 and 2). If B-cell preparations were sensitised (coated) with normal rabbit serum or RAMB antiserum followed by SARG-latex, no label was detectable on the surface (Fig. 1). However, surface immunoglobulin determinants could be visualised on 80% of the lymphocytes with SARG-coated atex if the cells were sensitised with RAMG (Fig. 2). Except

for the presence of label, there was no detectable difference between labelled and unlabelled cells. B lymphoyetes labelled for surface Ig had 50–150 densely distributed latex spheres attached to the surface. With specific labelling there were sometimes many free latex particles on the surrounding glass, probably due to shearing as above. The dense pattern of Ig labelling observed was different from the patchy and sparse distribution seen on rabbit peripheral blood lymphocytes (PBLs) which have quantitatively less surface Ig<sup>12</sup>. The unlabelled population of Ig-negative cells (20%) probably represented a null population or at least cells with insufficient surface Ig to be detected by this labelling technique.

Lymphocyte preparations enriched for T cells (eluted from nylon columns) were heterogeneous in surface topography. Between 70 and 80% were villous (Figs 3 and 4); the remaining 20-30% had smoother surfaces, some with few or no microvilli. Latex label could not be detected on T-cell-enriched preparations sensitised with normal rabbit serum or RAMG followed by SARG-latex (Fig. 3). Specific cell surface labelling, however, was visible on more than 90% of cells sensitised with RAMB (Fig. 4). The label was usually seen as distinct patches (1-2 μm across) containing 5-15 spheres. Most villous cells had quantitatively more surface label than smoother cells. Because differences in morphology may be due to cell preparations, for example, elution off a nylon wool column may induce formation microvilli in a subpopulation of T cells, we examined normal unfractionated BDF1 lymph node cells. Of these 30-40% were labelled with RAMB and SARG-latex. The morphology of the labelled cells was similar to that of the T-positive cells (90%) in the T-enriched population (that is, most of the labelled cells were villous), suggesting that nylon column fractionation did not affect significantly the surface architecture of the passed cells. The observed differences in T-cell morphology and labelling may represent different functional populations, especially since the more villous cell types had more T-specific surface label.

Cell preparation and processing for SEM may affect the surfaces of mouse lymphocytes. Rabbit PBLs are smooth in suspension by SEM and TEM, but develop microvilli and pseudopodia when adherent to glass for SEM<sup>13</sup>. In the study reported here all cells were allowed to adhere to glass for 30 min to produce conditions for villous formation. Environmental factors, including temperature<sup>14</sup>, cell preparation<sup>15</sup> and cell cycle<sup>16</sup> can affect the topology of the surface membrane. Thus these parameters must be evaluated before the SEM appearance of individual cells can be used for identification.

The controversy of 'hairy' B cells and 'smooth' T cells is unresolved. Contrary to our findings, with mouse lymphocytes,

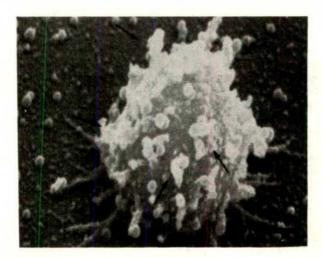


Fig. 4 BDF1 T cells sensitised with RAMB (T-cell-associated antigen). T lymphocytes treated with RAMB and SARG-latex (see text) showed specific surface labelling of brain associated (T-dependent) antigen. Latex label was visualised on 90% of the cells as distinct patches (arrows).

SEM observations of human lymphocytes showed that B cells usually had numerous microvillous projections, whereas T cells were smooth with relatively few microvilli3.4. In our experiments mouse B cells were relatively smooth, while most T cells had extensive microvilli and pseudopodia. We interpret these morphological distinctions with caution because there is usually a great deal of heterogeneity in surface architecture among a given population of cells. Even in our enriched population of T cells, a wide variety of positive labelled stubby and villous cells was found. Thus, we conclude that B or T cell identification by SEM is tenuous without immunospecific markers.

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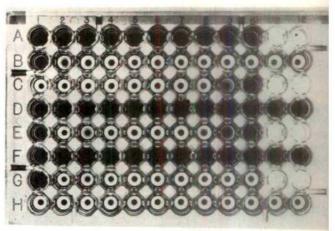
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# Haemagglutinins in commercial preparations of monosaccharides

We have found that several commercial preparations of monosaccharides possess haemagglutinating activity. This activity was observed with monosaccharide concentrations close to those usually used to inhibit the biological effects of lectins. The activity is not due to the monosaccharides themselves but is associated with a high molecular weight compound(s) active in some preparations of monosaccharides.

Soybean agglutinin was purified by affinity chromatography as described previously<sup>1</sup>. Human erythrocytes, drawn into EDTA and used within 7 d, were washed four times with phosphate buffered saline (PBS), pH 7.2-7.4, trypsinised as 4% (v/v) suspensions in PBS with 0.01% (w/v) crystalline trypsin (Sigma, type XI) for 90 min at 37° C and washed again



Agglutination of type O, trypsinised human erythrosuspensions in Microtiter wells) by soybean agglutinin and by N-acetyl-D-galactosamine and D-galactose. Four titrations of soybean agglutinin as serial twofold dilutions in PBS or PBS plus monosaccharide are shown. The soybean agglutinin concentration in the first well of each titration series (A-1, C-1, E-1, G-1) was 1,000 μg ml<sup>-1</sup>. The twenty-first and twenty-second wells of each titration (B-11 and B-12, D-11 and D-12, F-11 and F-12, H-11 and H-12) were controls and contained no soybean agglutinin. In the first titration (A-1 through B-10), no monosaccharide was present. The wells of the second (C-1 through D-10), third (E-1 through F-10) and fourth (G-1 through H-10) titrations of soybean agglutinin and the wells of their respective controls contained 0.01 M N-acetyl-p-galactosamine (Pfanstiehl, lot no. 8076A), 0.01 M p-galactose (Pfanstiehl, lot no. 9145), and 0.01 M p-galactose (Baker, lot no. 2-0411), respectively.

four times with PBS. Haemagglutination assays were performe as described previously2 in unperturbed wells of Microtite plates (Cooke Engineering Co.) with 1% (v/v) erythrocyt suspensions and twofold serial dilutions in PBS of the aggle tinin of interest. The sedimentation patterns of the 1% erythro cyte suspensions were read after 2 h at room temperature. positive pattern, indicating agglutination, is uniform effacement of the bottom of the well by erythrocytes; a negative pattern indicating no agglutination, is a circular clump of erythrocyte surrounded by a concentric, clear zone (Fig. 1).

Our initial observation was that some preparations inhibitory sugars produced positive haemagglutination pattern in Microtiter wells with concentrations of soybean agglutini insufficient for agglutination (Fig. 1). As expected, soybea agglutinin-mediated haemagglutination was inhibited N-acetyl-D-galactosamine and by two preparations of galactose. It was unexpected that two of the three inhibitor monosaccharides tested. N-acetyl-p-galactosamine (Pfanstieh lot no. 8076A) and p-galactose (Pfanstiehl, lot no. 9145), cause haemagglutination (wells C-10 through D-12 and E-10 through F-12, respectively) when the concentration of soybean aggle tinin was too low for hacmagglutination. No agglutination w produced by agglutinating preparations of N-acetyl-p-galacte samine or p-galactose in the presence of high concentrations soybean agglutinin (wells C-1 through C-8 and E-2 through E-8 Therefore, it seems that some interaction between soyber agglutinin and the agglutinating compounds present in the monosaccharide preparations prevented haemagglutination l the agglutinating compounds. The nature of this interaction not understood; the haemagglutinating material may bind soybean agglutinin. The interaction is obviously not inhibite by the parent monosaccharide solution. A similar phenomene was observed with p-mannose and concanavalin A (Mile Yeda).

Preparations of these and other monosaccharides we surveyed for haemagglutinating activity in the absence of lecti (Table 1). Since not all preparations of D-galactose an N-acetyl-p-galactosamine caused haemagglutination, we con clude that the haemagglutinating activity is not due to the

monosaccharides but must be produced by another compound active in some preparations.

We have investigated the nature of the haemagglutinating compound present in D-galactose (Pfanstiehl, lot no. 9145). Trypsinised erythrocytes were about ten times more susceptible to agglutination than were non-trypsinised erythrocytes; types A, B and O erythrocytes were equally agglutinable. Agglutination was not totally reversed by nine washes of the agglutinated erythrocytes with 100-fold volumes of PBS. Haemagglutinating activity was removed from a 0.2 M galactose solution by preincubation for 2 h at room temperature with 5 × 10<sup>7</sup> type O erythrocytes per ml. The haemagglutinating activity in the sugar solution was destroyed by boiling for 90 min; the activity was resistant to up to ten cycles of freezing and thawing.

The haemagglutinating activity in galactose (Pfanstiehl, lot no. 9145) was found in the retentate and could be concentrated quantitatively and washed free of monosaccharide by ultrafiltration over an XM-50 ultrafilter (Amicon) which nominally retains proteins of molecular weight greater than 50,000. The activity was nearly completely retained by an XM-300 ultrafilter that retains proteins of molecular weight greater than 300,000. The haemagglutinating activity in solutions of p-mannose (Baker, lot no. 1-9855) and D-glucose (Mallinckrodt, lot no. XDX) could be separated in a similar fashion.

Preliminary characterisation of the concentrated compound, washed free of D-galactose by ultrafiltration indicated that the compound had a high carbohydrate content by the phenolsuphuric acid assay3. A Lowry determination4 indicated a protein content equivalent to 90 µg of bovine serum albumin per mg of dried material, but the carbohydrate content may have interfered with the procedure5; preliminary analysis revealed only trace amounts of amino acids. Ultraviolet spectra of agglutinating preparations of unfiltered p-galactose (~ 0.2 M) show a peak at 280 nm (~ 0.25 absorbance units). The material responsible for the absorbance could be removed partially by ultrafiltration, however, without demonstrable loss of haemagglutinating activity. Possible sources of these high molecular weight haemagglutinating compounds are the

Pable 1 Survey of sugar preparations for haemagglutinating activity

a) Preparations with haemagglutinating activity	Y
	Lowest
	haemagglutinating
	concentration
	(m <b>M</b> )
N-acetyl-D-galactosamine	4
(Pfanstiehl 8076A)	
p-galactose (Pfanstiehl 9145)	4
p-galactose (P-L Biochemical 331261)	8
Galactose (Pfanstiehl 9638)	8 8 8
o-mannose (P-L Biochemical 332651)	
mannose (Calbiochem 34148)	16
)-mannose (Baker 1–9855)	16
o-mannose (Pfanstiehl 10012)	32
deoxy-D-glucose (Pfanstiehl 9466)	40
)-glucose (Mallinckrodt XDX)	160
o-fucose (Pfanstiehl 8742)	160
b) Preparations without haemagglutinating Activity	
actose (Pfanstiehl 9471)	
N-acetyl-p-galactosamine (Pfanstiehl 9918)	
-galactosamine hydrochloride (Pfanstiehl 9003)	1
3-galactose (Baker 2-4011)	
Galactose (Fisher 793901)	
N-acetyl-D-glucosamine (Pfanstiehl 9245)	
Methyl-α-D-mannopyranoside (Pfanstiehl 9375 a	and 9589)
373 a-arabinose (Pfanstiehl 9288ML)	*****

Haemagglutinating activity was assayed in Microtiter plates with a 1% suspension of trypsinised, type A erythrocytes and serial twofold dilutions of the monosaccharides in PBS as described in the text. The lowest millimolar concentrations of monosaccharides producing haemagglutination are shown. The highest concentration tested was 160 mM. The sources and lot numbers are indicated in parentheses.

-fucose (Pfanstiehl 9698ML)

lucrose (Baker 30388)

biological source of the sugar (for example, ivory-nut meal for mannose6), biological agents used during production of the sugar (for example, bakers' yeast for galactose7), and polymerised products of sugars and/or sugar derivatives8 formed during manufacture and purification of the sugar. Heatpolymerised furfurals8 do not appear to be responsible for the haemagglutinating activity as atmospheric refluxing of aqueous solutions of galactose and glucose sufficient to produce intense ultraviolet absorption at 280 nm did not produce haemagglutinating activity. We conclude tentatively that the haemagglutinating material obtained from galactose is a heat labile macromolecule composed primarily of carbohydrate with, perhaps, a minor protein component.

The presence in many monosaccharide preparations of a large molecular weight compound with haemagglutinating activity may have important consequences for several kinds of studies on the biological effects of monosaccharides, and for studies of any biological system containing or utilising monosaccharides. Such considerations are of obvious importance in tissue culture where glucose is a component of culture media and in studies involving the effects of monosaccharides on tissue culture cells and intact organisms as, for example, in sugar transport9. The presence of these compounds in monosaccharides may affect sugar-dependent characterisation of the physical properties and biological specificities of lectins and even the purification of lectins by affinity techniques utilising monosaccharides. Until the properties of these high molecular weight compounds are more fully understood, ultrafiltration can be used for complete removal of the high molecular weight compounds and the associated haemagglutinating activity from the monosaccharides.

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## Molecular and crystal structure of deoxyguanosine 5'-phosphate

RECENT crystallographic studies of the dinucleosides ApU (ref. 1) and GpC (ref. 2) have given experimental proof for the base pairing arrangement proposed by Watson and Crick for the DNA double helix3. Another striking feature of this structure relates to the torsional angle about the C5'-C4' bond in the phosphate-sugar backbone chain. In the Crick and

Watson model4, this conformation is gauche-trans (GT). Crystal structures of 5'-nucleotides, dinucleosides and dinucleotides so far studied, however, have shown only the gauchegauche (GG) conformation about this bond. The GG conformer is also the only one found in the refined models of the proposed structure of the double helical nucleic acids and polynucleotides<sup>5-7</sup>. The only nucleotide with a GT conformation is 6-azauridine-5'-phosphate8 which is not a normal monomer unit of nucleic acids. It is also reported that 5'-dGMP assumes preferentially GT conformation in solution9.

We wish to report here the molecular structure of the DNA monomer unit, deoxyguanydilic acid (dGMP-5') as determined by a complete three-dimensional X-ray structure analysis. The nucleotide shows the GT conformation about the C-5'-C-4' bond similar to that occurring in the Crick and Watson model.

Crystals of the disodium salt of deoxyguanosine 5'-phosphate were grown by slow diffusion of alcohol into aqueous solutions of the compound<sup>10</sup>. Thin platty crystals about 2 mm long were obtained in about 3 week. The crystal belongs to monoclinic system with space group P2<sub>1</sub>. The unit cell dimensions are a = 16.00 Å; b = 10.73 Å; c = 5.575 Å;  $\beta = 101.9^{\circ}$ ;  $d_{obs} = 1.64$  g cm<sup>-2</sup>;  $d_{cal} = 1.64$  g cm<sup>-2</sup>; Z = 2. Density measurements carried out using carbon tertachloride-bromoform mixtures indicated the presence of four water molecules in the asymmetric unit. Intensity data were collected by multiple film, equiinclination, Weissenberg technique for hO1-h91 and hkO layers using CuK, radiation. The data were measured visually by comparison with precalibrated graded intensity scale. A three-dimensional Patterson function was computed and the phosphorous atom was located from the Harker section. The structure was solved by symbolic addition

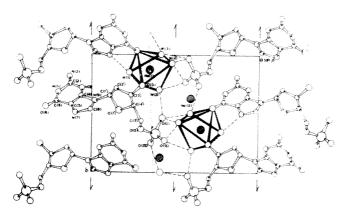


Fig. 1 Structure of dGMP-5' Na<sub>2</sub> 4H<sub>2</sub>O viewed down c axis. One of the sodium atoms (Na(1)) has an octahedral coordination shown by thick lines. Hydrogen bonds are indicated by broken lines. The stacking pattern of the bases belonging to molecules related by a 'c' cell translation is not shown for the sake of clarity.

method. Phases for 290 reflections were obtained using the program 'Multan'11. An E map was computed for the best set which gave a Karle R factor for 28.59%. The entire molecule with two sodium ions and one water oxygen atom were identified from the E map from a knowledge of the position of phosphorous atom and by model building approach. The current structure has an R factor of 9.0%. The view of the structure down c axis is given in Fig. 1.

The glycosidic torsional angle  $\chi_{CN}$  is 52.6°. The relative orientation of the base with respect to the sugar is therefore, anti. The  $\chi_{CN}$  value is within the range expected of  $\beta$ -purine nucleotides. The best four atom plane for the deoxyribose moiety passes through C1'-C2'-C3'-C4'. The O1' atom shows an endo puckering, being displaced by 0.6 Å from this plane in striking contrast with the other three DNA monomers where the furanose ring is puckered either with C2' or C3' (refs 12-14).

The conformation about the C5'-C4' bond is GT with

 $\phi_{\mathbf{O5'}-\mathbf{O1'}}=62.5^{\circ}$  and  $\phi_{\mathbf{O5'}-\mathbf{C3'}}=174.8^{\circ}$ . As mentioned earlie this is the first structure to show this conformation which i close to that found in Watson-Crick DNA model ( $\phi_{ns} = 38$  $\phi_{oc}=155^{\circ}$ ).

In model building studies and refinement on nucleic act structures one tends to use the structural parameters an torsional angles within the constraints prescribed by the result of single crystal X-ray studies on flucleic acid constituents. I seems to us that the conformational features observed i dGMP-5' have also to be considered in possible polynucleotid conformations and foldings particularly of the DNA. Th GT conformer and the O1' endo puckering of sugar ring are als of direct relevance to the concept of rigid nucleotide unit25.

We thank Professor V. S. Venkatasubramanian for hi interest and Dr K. Venkatesan and Mr S. Ramakumar fo providing the modified version of the 'Multan' program T.P.S. thanks the UGC authorities for financial support. Note added in proof:- An accurate independent X-ray analysis of this nucleotide has now been reported by Young, Tolli and Wilson<sup>16,17</sup>. Our findings are in close agreement with th

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#### Errata

In the article "Volatilisation of mercury and organomer curials determined by inducible R-factor systems in entering bacteria" by J. Schottel et al. (Nature, 251, 335; 1974), it the last two lines of the legend to Fig. 1 and also in the penultimate line of para. 3, J53(471a) should read J53(R471a) on each occasion.

In the article "Subunit structure of chromatin" by M. No. (Nature, 251, 249; 1974) line 7 of the legend to Fig. should read '... and  $V_m=9.2$  ml in 0.2 mM ... ' and no as originally printed.

# matters arising

### Do molecular biologists come of age in Aries?

WINDSOR has concluded that, "More molecular biologists were born under the sign of Aries than any other sign. More taxonomists were born under the sign of Cancer than any other sign and relatively few were born under Scorpio." Lest acceptance of these findings induces prospective parents to time the birth of their offspring to maximise their chances of becoming molecular or taxonomic biologists, a modest statistical analysis of Windsor's data seems in order

Table 1 contains the frequencies, arranged by Sun sign and biological bent, of the birthdates of 812 biologists in the sample observed by Windsor1. The relative frequencies of births for the general population of the United States for 1934 by Sun sign were estimated by adding the products of the monthly relative frequencies by the respective fraction of each of the two months contributing to a given Sun sign interval as given by Parker and Parker<sup>2</sup>.

In the  $\chi^2$  tests for goodness of fit to the data in Table 1, the critical value is  $\chi^2_{0.05,11} = 19.675$  if  $P \le 0.05$  is the signicance level to reject the null hypothesis, Ho: that each sample has the same frequencies as the general population. Since  $0.75 \le P(\chi^2 \ge 7.282) \le 0.90$  for the taxonomists,  $0.10 \le P(\chi^2 \ge 16.368)$ ≤ 0.25 for the molecular biologists, and  $0.25 \le P(\chi^2 \ge 11.580) \le 0.50$  for the combined sample of 812 biologists, the null hypotheses cannot be rejected. That the samples of taxonomists and molecular biologists could have come from the same population is confirmed by the heterogeneity  $\chi^2$  test:  $0.25 \le P(\chi^2 \ge 12.070) \le$ 0.50.

As might be expected, the same conclusion that the biological discipline of the 812 scientists is independent of the Sun sign of birth is borne out by a  $2 \times 12$ contingency table3,4 which involves no assumptions about the birth frequencies per Sun sign for the general population. In this case,  $0.25 \le P(\chi_{11}^2 \ge 11.495) \le$ 0.50.

Even if the  $\chi^2$  tests had permitted the rejection of the null hypotheses, the validity of Windsor's conclusions would have been restricted to biologists in American men and women of science<sup>5</sup> whose birthdays were listed. Other factors such as age and sex proportions among axonomists, molecular biologists, and the general population would have complicated analysis and interpretation even further.

Table 1 Frequencies (f) of birthdates by Sun sign from a sample of 812 biologists<sup>1</sup> and expected frequencies (F) if the proportions were the same as in the general population (see text)

Sun sign	General population	Taxo	onomists	Molecula	ar biologists	Cu	ımulative
	%	f	F	f	F	f	F
Aquarius	9.05	<b>26</b>	30.98	35	42.57	61	73.55
Pisces	8.18	31	28.00	36	38.48	67	66.48
Aries	8.11	28	27.76	58	38.15	86	65.91
Taurus	8.46	30	28.96	32	39.80	62	68.76
Gemini	8.08	31	27.66	39	38.01	70	65.67
Cancer	8.84	38	30.26	41	41.59	79	71.85
Leo	• 8.67	32	29.68	32	40.79	64	70.46
Virgo	8.98	31	30.74	42	42.24	73	72.98
Libra	8.17	25	27.97	41	38.43	66	66.40
Scorpio	7.98	18	27.32	41	37.54	59	64.86
Sagittarius	7.75	27	26.53	40	36.46	67	62.99
Capricorn	7.64	25	26.15	33	35.94	58	62.09
Total	99.91	342	342.00	470	470.00	812	812.00
χ²			282	16.	.368	11	.580

While I hope that this modest analysis will be instructive and entertaining to studients of biostatistics, I regret that astrologers may be disappointed, although they usually stress2 that birth place and planets' positions are the determining factors of the birth chart or 'horoscope' used to suggest the likelihood of certain

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Windsor, D. A., Nature, 248, 788 (1974). Parker, D., and Parker, J., The complete astrologer's sun-signs guide, 5-8 (Crown Publishers, New York, 1973). <sup>3</sup> Zar, J. H., Biostatistical analysis, 41-69

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5 American men and women of science: the physical and biological sciences, twelfth ed. (edit. by Jacques Cattell Press), 1-6, (Bowker, New York, 1971-73).

DR WINDSOR REPLIES-Dr Colás certainly provides an appropriate criticism of my Sun sign findings.

The statistical method I used, before publication, was a non-parametric analysis of a manifold population as described by Tate and Clelland<sup>1</sup>. Considering the 12 Sun signs as a manifold population, the hypothesis tested was that the distribution of birthdays was uniform,  $H_0 = Ar = T$ = G ... = P = 1/12.  $\chi^2 = [(f^0 - f^e)^2]/f^e$ , where  $f^0$  is the frequency of characters in the sample and  $f^{e}$  is the frequency expected under the null hypothesis, that is, the average.

 $\chi^2 = [(Ar - f^e)^2]/f^e + [(T - f^e)^2]/f^e +$  $[(G-f^e)^2]/f^e ... + [(P-f^e)^2]/f^e.$  $\chi^2$  for molecular biologists = 13.818;  $\chi^2$ for taxonomists = 8.666. At 11 degrees of freedom the probabilities of the hypotheses being true are 0.025 and 0.600, respectively. Since the hypothesis is rejected in both cases, therefore, the distributions were not random.

I compared molecular biologists with themselves and I compared taxonomists with themselves. I did not compare them with each other or with the general population. Molecular biologists and taxonomists are simply different kinds of biologists and must be considered independently or else the distinction is lost. Comparing them with the general population is unnecessary because it is obvious that, for the most part, they did indeed come from the same general population. The problem is not to identify similarities and establish origins, but to separate components and identify their characteristics. I used American men and women of science as my data base because it is a readily accessible, reproducible sample. Its validity can only be determined by gathering additional data. To cite one instance of possible invalidity, many practising molecular biologists are probably not listed as such, but appear classified as biochemists or physiologists, since the scientists describe their own disciplines.

My data were, admittedly, meagre, but it was hoped that their publication would inspire, or even goad, scientists with more resources to pursue this line of research by acquiring and analysing larger data bases. The basic concept of extraterrestrial influences on human activities is so important that it certainly deserves much more investigation than is now being performed.

PO Box 604. Norwich, New York 13815.

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# Ascorbic acid and nitrosamines

EDGAR<sup>1</sup> has proposed that ascorbic acid might inhibit the carcinogenic action of nitrosamines and other carcinogens that may act by alkylation. The rationale was that ascorbate might be alkylated *in vivo* before the carcinogens can react with cell macromolecules. The experimental basis for Edgar's thesis was the statement by Kamm *et al.*<sup>2</sup> that ascorbate inhibited the liver necrosis induced by dimethylnitrosamine (DMN). This statement, however, was presented without experimental details and was subsequently withdrawn<sup>3</sup>.

The main concern of the study by Kamm et al. was the inhibition by ascorbate of the liver toxicity induced by oral administration of aminopyrine plus nitrite. This study followed our report in 1972 suggesting, on the basis of in vitro experiments, that ascorbate might be used to block in vivo formation of Nnitroso compounds from nitrosatable chemicals (for example, drugs), since ascorbate efficiently reduces nitrite4. Subsequently, the report by Kamm et al.4 appeared. Greenblatt<sup>5</sup> found similar results in mice to those of Kamm et al., but stated that ascorbate did not affect DMN toxicity. We found that ascorbate prevented liver damage from gavage of dimethylamine plus nitrite to rats and, from experiments presented in detail, that ascorbate did not significantly affect the production by DMN of liver necrosis and elevated serum transaminase levels6 Ascorbate did not affect transplacental carcinogenesis in rats by ethylnitrosourea, but inhibited carcinogenesis by ethylurea plus nitrite7.

We are concerned that our original suggestion should not be extended without basis to the hypothesis that ascorbate might have a much wider inhibitory action on carcinogens. The interesting suggestion of Edgar is not supported by the results reviewed here, which were mostly made public after Edgar's paper was submitted.

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Edgar, J. A., *Nature*, **248**, 136–137 (1974).
 Kamm, J. J., Dashman, T., Conney, A.

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<sup>7</sup> Ivankovic, S., Preussmann, R., Schmähl, D., and Zeller, J., in N-Nitroso Compounds in the Environment (edit. by Bogovski, P., Walker, E. A., and Davis, W.) (International Agency Research Against Cancer, Lyon, in the press).

• Nature regrets that when this article was first published (250, 684; 1974) it contained certain typographical errors which may have caused confusion.

Scrapie

In a group of Herdwick sheep genetically selected for susceptibility to subcutaneous injection with SSBP/1 scrapie brain pool, Pattison reported¹ the occurrence of two cases in uninjected animals. He concluded "... that these two cases arose spontaneously by genetic selection". The simplest explanation of these two cases, however, is that they were due to infection with scrapie from some unrecognised source with later cases resulting from lateral and maternal transmission of the infection.

The factual parts of Pattison's report are in accord with our own findings. Cheviot sheep have been selected successfully for increased or decreased susceptibility to scrapie2,3 following subcutaneous injection with the same source of agent as that used by Pattison. These Cheviot sheep were bred on a geographically isolated farm, away from known cases of scrapie and for the first 7 yr no natural cases of scrapie occurred. Since 1968, however, when a natural 2-yr-old case occurred in the positive selection line, the incidence of natural cases has built up as shown in Table 1, and 81 of the 83 cases have occurred in the positive line. The other two cases have occurred recently in a group of 11 positive-line × negativeline crosses born in 1970. As in Pattison's cases these natural Cheviot cases are

easy to distinguish on histological criteria. Because many different strains of scrapic agent can be recognised by the characteristic type, severity and distribution of brain lesions which they produce<sup>4</sup>, the histological difference found in the sheep is direct evidence that no component of SSBP/I is involved in the natural Cheviot cases and this is supported by a failure so far to isolate from them either 22A, 22L or 22C (three strains of agent known to be present in the SSBP/I pool<sup>5</sup>).

The simplest explanation for the occurrence of the natural Cheviot and Herdwick cases is that the necessary conditions for the total 'isolation' of a flock from direct or indirect contact with scrapie agent are not yet understood. This is not surprising in view of the well known extreme resistance of scrapie agents to physical or chemical inactivation. Also, Icelandic evidence supports the view that there can either be long term persistance of scrapie infectivity on farms in the absence of sheep or that some vector is involved (ref. 6 and P. A. Palsson and B. Sigurdsson, unpublished). Quite apart from these considerations scrapie is known, quite definitely, to be naturally transmitted both laterally and maternally in field conditions (refs 7 and 8 and J. L. Hourrigan et al., unpublished) and among housed sheep<sup>8-12</sup>.

One comment is fundamental; is should not be assumed that the Herdwick; and Cheviots which are susceptible to peripheral injection with SSBP/1 scrapic will be susceptible to all strains of scrapic or that the resistant lines will be resistant to all strains. Such an assumption is at the basis of Pattison interpretation. The situation is clearly illustrated by work with scrapic in mice where it is not possible to describe on genotype as susceptible and another a resistant unless the strain of scrapic agent dose of agent and route of infection are specified 13.

Although the work with the Chevic sheep is less advanced than that in mice there is evidence that a similar situatio applies in both species. Pattison extes hi own report of scrapic infectivity bein produced from apparently normal mous tissue: these results were interpreted bhim as evidence for spontaneous generation of scrapic infectivity ('unmasking

Table 1 Incidence (%) of natural scrapie in two lines of Cheviot sheep selected for susceptibili (positive line) or resistance (negative line) to subcutaneous injection with SSBP/1 scrapie bra pool

				ekernese (marries in Cityania	mariet aminor recorded	and the second	To the same of
Year born	1957-60	1961-65	1966	1967	1968	1969	1970
The state of the s	0						
Foundation stock	U	2000	100000	-	G-MONT	- Name Com	
Selection   f positive	ALCONO.	0	1	7	37	23	391
line negative	and the second	0	0	0	0	0	01
							- 1

The flock size varied from 120-200 ewes and there were approximately equal numbers in the two selection lines.

<sup>&</sup>lt;sup>2</sup> Kamm, J. J., Dashman, T., Conney, A. H., and Burns, J. J., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 747-749 (1973).

<sup>&</sup>lt;sup>3</sup> Kamm, J. J., Dashman, T., Conney, A. H., and Burns, J. J., in N-Nitroso Compounds in the Environment (edit. by Bogovski, P., Walker, E. A., and Davis, W.) (International Agency Research Against Cancer, Lyon, in the press).

<sup>&</sup>lt;sup>4</sup> Mirvish, S. S., Wallcave, L., Eagen, M., and Shubik, P., Science, 177, 65-68 (1972).

but by others as being more likely to be due to accidental contamination <sup>14</sup>. If contamination is the correct explanation then the 'spontaneous' agent should be identical with one of the small number of strains in use in Pattison's laboratory but material has not been available for agent identification. Pattison also cites the work and interpretations of Parry<sup>15</sup> but fails to quote the critical reanalysis<sup>16</sup> of Parry's data which produced entirely different conclusions.

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### Ageing cell cultures

Is there an irreversible loss of mitotic ability in mammalian cell populations during ageing<sup>1</sup>? The results of continuous labelling alone can neither confirm nor refute this hypothesis.

Macieira-Coelho<sup>2</sup> continuously labelled cultures of human embryonic fibroblasts with tritiated thymidine. He found that the proportion of unlabelled cells declined to zero in a convex fashion. His result is an inevitable consequence of the continuous labelling method.

Macieira-Coelho did not distinguish between unlabelled dividing cells and unlabelled non-dividing cells. By definition non-dividing cells will not take up label and will not reproduce. If the initial proportion of unlabelled nondividing cells is  $q/2^{\circ}$ , then after k population doublings this proportion will be reduced to  $q/2^k$ . The proportion of unlabelled dividing cells will decline at a faster rate since the number of dividing cells that are unlabelled also will decrease. The proportion of unlabelled cells is the sum of these two proportions. It will decline to zero in a convex fashion (Table 1).

It will decline whether time is measured, chronologically, in population doublings, in split ratios or in cell generations<sup>3</sup>. It will decline whether the population is growing or static, for in a homeostatic population, where cell death is balanced by cell birth, the number of unlabelled cells will decline. It will decline whether or not un-

labelled cells continue to enter the division cycle.

The results reported by Macieira-Coelho<sup>2</sup> are artifacts of the continuous labelling method.

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- Good, P. I., and Watson, D., Exp. Geront., 8, 147 (1973).
- <sup>2</sup> Macieira-Coelho, A., *Nature*, **248**, 421 (1974).
- <sup>3</sup> Good, P. I., Cell Tissue Kinet., 5, 319 (1972).
- <sup>4</sup> Smith, J., and Hayflick, L., J. Cell Biol., 62, 48 (1974).

DR MACIEIRA-COELHO REPLIES—I think the theoretical speculations made by Dr Good are more likely to be artifacts than the experimental results on which I have based my conclusions.

Dr Good says that the decline in the population of unlabelled cells is an inevitable consequence of the continuous labelling method. As a matter of fact, my data show very clearly that this is not the case, and that the proportion of unlabelled cells depends on the size of the inoculum. With high inocula not all the cells have time to enter the cycle before division stops due to cell crowding. Furthermore, if after a continuous labelling one finds only 6% of unlabelled cells, regardless of how this equilibrium is reached at resting phase (loss of non-dividers, dilution, and so on) it means that there are only 6% of cells which did not enter the S period. The possibility remains that these cells are slowly cycling cells delayed in other periods, a hypothesis most mathematicians do not like to consider.

Eight years ago I published results' which led to the conclusion that during the growth decline of human fibroblasts the cell population becomes strongly heterogeneous and the cells show a spectrum between the two extremes, that is, complete inhibition of division and the normal division cycle. What has to be kept in mind is the presence of a spectrum, and the experimental evidence suggests that there is no predominant cell type (that is, cells with fast, intermediate or long generation times, or non-dividers). If there is a fraction of cells which do

not divide at all in the population, this is a small fraction as my data have now shown. There is no reason whatsoever to make speculations about growth kinetics stating that this small fraction would play a bigger role than the rest of the spectrum of cells with a whole range of generation times.

<sup>1</sup> Macieira-Coelho, A., Ponten, J., and Philipsen, L., Expl Cell Res., **42**, 673 (1966).

### Intelligence and handedness

GIBSON¹ has stated that his data do not support Levy's² contention that left-handed subjects have significantly lower visuospatial IQ's. Cohen's³ factorial study of the Weschler adult intelligence scale suggests that a more appropriate test of verbal IQ than the full Weschler scales used by both Gibson and Levy would consist of the average of age-weighted scores on subtests weighting on verbal comprehension (information, comprehension, similarities, vocabulary) and a test of visuospatial IQ would consist of subtests weighting on perceptual organisation (block design, object assembly).

Table 1 Comparison of two measures Right handed handed 125.5 7.08 mean 123.3 Verbal IQ s. d. 5 36 112.7 mean 114.6 Performance IQ 6.72 6.82 s. d. Verbal comprehension 14.78 score mean 1.37 1.27 s. d. Perceptual organisation 12 37 12.4 mean score 3.54 s.d. 1.60

Comparing a small sample of left- and right-handed subjects, ten of each group, no significant differences between the two groups were found on either the full verbal and performance IQ's or on the verbal comprehension and perceptual organisation scores (Table 1). These data support Gibson's findings.

L. D. ROBERTS

Department of Psychology, University of Bradford, Bradford, Yorkshire BD7 1DP, UK

- Gibson, J. B., Nature, 243, 482 (1973).
- <sup>2</sup> Levy, J., *Nature*, **224**, 614 (1969).
  <sup>3</sup> Cohen, J., *J. Consult. Psychol.*, **21**, 451

DR GIBSON REPLIES—A reanalysis of my data along the lines suggested by Roberts has given similar results. There are no significant differences between left-handed and right-handed subjects on either the verbal comprehension or perceptual organisation scores.

University of Cambridge

 Percentage of unlabelled cells at confluence, by type, following continuous exposure to labelled tritiated thymidine

	to labolita iii		
	Observed <sup>2</sup>	Theore	etical
	00001100	Non-dividers	Dividers
Split Ratio	%	%	%
No transfer	100	16 (ref. 4)	84 (ref. 4)
1:1	79	16	63
1:2	25	8	17
1:4	3	4	
	1:1 1:2	Observed <sup>2</sup> Split Ratio % No transfer 100 1:1 79 1:2 25	Non-dividers   Split Ratio   %   %   %   %   %   %   %   %   %

No correction was made in this table for cell death or cell loss during transfer3.

### Classified Advertisements

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discretion and without explanation. All advertisements must comply with the British Code of Advertising Practice.

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#### APPOINTMENTS VACANT

#### ASSISTANT PROFESSOR IN **PALEONTOLOGY** UNIVERSITY OF TORONTO

Ph.D. required from July 1, 1975, with teaching and research interests in invertebrate paleontology or micro-paleontology and its stratigraphic applications. Duties include undergraduate and graduate teaching, development and maintenance of a research programme, and supervision of graduate students. Salary dependent on qualifications and experience. Address enquiries to Dr. D. W. Strangway, Chairman, Dept. of Geology, University of Toronto, Toronto, Ontario MSS 1A1, Canada, (1591)

#### UCLA

University of California, Los Angeles, Department of Geology invites applications for two-year faculty appointments as assistant professor. A Ph.D. is required. Two faculty two-year positions will be available beginning September 1975. Applicants must have truly superior capabilities in one or more of the relevant basic physical or biological sciences. Two earth scientists will be selected from the three following general areas:

(1) Those engaged in research in the content of the relevant basic physical or biological sciences.

(1) Those engaged in research in one or more of the fields (a) igneous or metamorphic petrology; (b) ore genesis; (c) experimental or theoretical geochemistry; (d) mineral physics. Examples of emphasis include thermodynamics of irreversible processes and chemical or thermal diffusion.

(2) Those engaged in research in the areas of tectonophysics, geological physics, or structural geology. Of particular interest are: (a) solid-state aspects of microscopic phenomena-fracture and flow of rocks, diffusion, thermodynamics, and lattice dynamics; (b) global tectonics; (c) quantitative modelling of large or small scale processes.

(3) Marine science, those engaged in research in organic geochemistry, ecology, or marine geology. Examples of emphasis include diagenesis of sediments, interstitial water, evolution, phycology (calcarcoss algae), invertebrate biology, and global tectunies. tectonics

Clarence A. Hall, Chairman
Department of Geology
University of California Los Angeles
Los Angeles, California 90024

Minority and ethnic earth scientists are encouraged to apply. UCLA is an affirmative-action/equal opportunity employer. (1654)

#### UNIVERSITY OF LIVERPOOL CHAIR OF HISTOLOGY AND CELL BIOLOGY (MEDICAL)

Applications are invited for the Chair of Histology and Cell Biology (Medical), within the Faculty of Medicine, which will become vacant on April 30, 1975, following the retirement of Professor N. M. Hancox, It is not a requirement that the holder of the Chair should possess a medical qualification.

the Chair should possess a medical qualification.

The salary will be within the range approved for full-time professorial appointments and in any case not less than £6.264 per annum.

Applications (12 copics), together with the names of three referees, should be treceived not later than November 29, 1974 by the undersigned, from whom further particulars may be obtained. (Candidates overseas may send one copy only, by airmail). Quote ref. RV/N/300.

H. H. Burchnall,

H. H. Burchnall, Registrar. (1749)

### LEICESTERSHIRE AREA HEALTH AUTHORITY (T) AREA MEDICAL PHYSICS DEPARTMENT

Applications are invited from Graduates for a newly established post in the Leicestershire Medical Physics Department. This department which is undergoing considerable expansion, provides a service for the

# **Electronic Engineer or Physicist** Senior or Basic Grade

A vacancy exists for an experienced Physicist or Electronic Engineer to set up an Audiological testing section involving clinical work,

This post will provide scope for developmental work in which the successful applicant will be encouraged to participate. Opportunities will occur for him to be engaged also in other aspects of Medical Physics at the Leicester Royal Infirmary.

Applicants should have a good honours degree in electronic engineering or physics with a special interest in electronics and experience in this field would be desirable, though not essential.

Salary on scale: Senior £3,345 to £4,377 p.a. • Basic Grade £2,046 to £2,994 p.a.

Further information from the Principal Physicist in Charge,

Applications stating age, experience and qualifications with the names two referees to the Hospital Secretary, The Leicester Royal Infirmary, Infirmary Square, Leicester.

Closing date for applications (November 18, 1974).

(1758)

# MINE GEOLOGIST

Applications are invited for the post of Mine Geologist to serve a group of Collieries in the Western Area of the National Coal Board. The successful candidate would be responsible for recording geological information and participating in investigations into the effects of geological environment on mining operations. The work will mainly involve underground duties.

Applicants should have an Honours Degree in Geology. Training in underground geological techniques will be given to the successful candidate.

Starting salary, in accordance with qualifications and experience, within the scale £2,345 to £2,885.

Write for application form to Area Staff Manager, National Coal Board, Staffordshire House, Berry Hill Road, Stoke-on-Trent. (1795)

Ministry of Overseas Development Centre for Overseas Pest Research, London

- To work on the identification and determination of pesticides in samples of water, soils, crops and animals collected in overseas
- □ Degree, HNC or equivalent in Chemistry □ Experience in Chromatography or pesticide residue analysis desirable 
  Age under 30 
  Appointment
- as Higher Scientific Officer (over £2800 to around £3750) or Scientific Officer (over £1950 to around £3050)  $\square$  Ref: SA/21/JA.  $\square$  Application forms (for return by 28 November 1974), from Miss M B McKiernan, Ministry of Overseas Development, Centre for Overseas Pess. Research, College House, Wrights Lane, London W8 58J.



(1834)

### NATIONAL COUNCIL FOR SCIENTIFIC RESEARCH

Applications are invited from suitably qualified persons for the post of:

# TREE PHYSIOLOGIST

Applicants must have a postgraduate degree or experience in tree physiology and research experience in decays of timber or decay in standing trees. Experience in tropical pines and eucalypts also desirable.

Excellent laboratory facilities and equipment exist to carry out physiological research. The appointee will be required to carry out research on the physiology of tropical pines and eucalypts of economic importance in Zambian forestry.

Salary according to qualifications and experience but a higher salary would be offered to exceptionally well-qualified and experienced senior scientists.

Professional Officers
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K3,240 by K240 to K4,440 K4,680 by K240 to K5,640

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K5,840 by K240 to K6,800

For Zambians there is a superannuation scheme. Non-Zambians will be paid a gratuity of 25 per cent of aggregate basic salary earned during service of not less than thirty months.

Accommodation will be provided on an economic rental basis but not exceeding 10 per cent of basic salary. Hard furniture will be provided. For Non-Zambians travel and educational allowances are available for minor dependent children attending school outside Zambia.

Applications giving full personal details, qualifications, experience and naming three referees should be submitted to:

The Secretary-General, National Council for Scientific Research, P.O. Box CH. 158, Chelston, LUSAKA, Zambia.

(1762)

#### **BIOCHEMIST**

Ph.D. or equivalent. Must have laboratory experience in vitamin D chemistry or action. To establish a vitamin D-focused laboratory programme in conjunction with university calcium researchers. Position at Research Institute. The Hospital for Sick Children, Toronto, with faculty appointment in Department of Biochemistry, University of Toronto. Salary commensurate with experience and qualifications. Apply before December 1st enclosing curriculum vitae and names of two referes, to:—The Secretary, Research Institute, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, M5G 1X8, Canado. (1661)

# UNIVERSITY OF TEXAS MARINE SCIENCE INSTITUTE

MARINE SCIENCE INSTITUTE

The University of Texas Marine Science Institute at Port Aransas is seeking applicants for an appointment, preferably at the ASSISTANT PROFESSOR level, from marine biologists capable of teaching courses in invertebrate zoology of marine organisms and of doing research in the area of physiological ecology. Applicants must have a strong research interest and a commitment to graduate training. Send curriculum vitae and a brief statement of research interests to the Director, The University of Texas Marine Science Institute, Port Aransas, Texas 78373. Affirmative Action/Equal Opportunity Employer. (1726)

#### UNIVERSITY OF GUELPH DEPARTMENT OF CHEMISTRY FACULTY POSITION

Applications are invited for a faculty position in the area of biochemistry or biophysical chemistry at the rank of assistant or associate professor, to begin September 1, 1975. Candidates must have a Ph.D. degree in chemistry or biochemistry and at least one year of postdoctoral experience in biochemistry or biophysical chemistry. Duties will include undergraduate and graduate teaching in biochemistry, supervision of graduate research, and independent research. Salary and rank will depend on qualifications and experience.

Applicants should provide a full curriculum vitae, transcripts of academic record, a brief description of research interests and a research proposal, and the names of three referees to A. K. Colter, Chairman, Department of Chemistry, University of Guelph, Guelph, Ontario, Canada, NIG 2WI. (1750)

# nature

A vacancy will arise soon in the Washington office of *Nature* for the important post of Assistant Editor responsible for supervising the refereeing of biological manuscripts submitted to the Washington office. A period of training in the London office under the supervision of the Biological Manuscripts Editor will precede the posting to Washington. The successful applicant may expect to be in Washington for not less than two years.

This job is an outstanding opportunity for a recently qualified British or American Ph.D who has a strong interest in ensuring the high quality of the biological papers we publish.

Apply with curriculum vitae and the names of two referees to:-

The Editor,
Nature,
Macmillan Journals Ltd.,
4 Little Essex Street,
London, WC2R 3LF

by November 15th

#### INSTITUTE OF GENETICS UNIVERSITY OF COLOGNE

#### POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for a postdoctoral research assistantship sponsored by the Ger-man Research Association to study chromoman Research Association to study characteristics and expression of gene activity in cultured mammalian cells in collaboration with Dr. Klaus Willecke. The appointment is tenable for two years, commencing January 1, 1975. The salary will be about 5,000 per annum (salary of a married person, 27 years old). Applications consisting of a brief curriculum vitae and the names of two referees should be sent to Dr. Klaus Willecke, Institut für Genetik, D 5 Köln 41, (1753)Weyertal 121, West Germany,

#### UNIVERSITY OF VICTORIA DEPARTMENT OF BIOLOGY

Applications are invited for a continuing position to teach and organize the Department's introductory biology course. The appointment will be made at the Assistant Professor level, although applicants with exceptional experience and qualifications must hold a doctorate degree, have previous experience and a special interest in teaching introductory biology, and be active scholars in their field of research. The area of specialized research is of less consequence than the applicant's abilities as a teacher, although a field complementing the Department's specializations in marine science or field biology is desirable. Teaching duties commence in September, 1975, and the closing date for applications is December 31, 1974.

Applications should include a curriculum vitae

Applications should include a curriculum vitae and the names of three referees. Applications should be directed to Dr. Michael Ashwood-Smith, Chairman, Department of Biology, University of Victoria, Victoria, B.C., Canada, V8W 2Y2.

(1766)

### INSTITUTE OF TERRESTRIAL **ECOLOGY**

# ANIMAL **ECOLOGIST**

The Institute of Terrestrial Ecology requires an experienced ecologist to work on behavioural ecology of Vertebrates and, as a first task, to develop research on mammalan predators. He/she, will be a member of a group of ecologists based at Banchory, Kincardineshire, and be expected to provide expertise in behaviour studies and to guide other staff.

Candidates should have several years experience of research, preferably on vertebrate predators in more than one habitat, and a wide interest in the ecology of vertebrates and their prey, including birds as well as mammals.

#### **OUALIFICATIONS**

An appropriate first or second class honours degree (or equivalent) and at least four years relevant post-graduate experience.

Appointment will be to the Senior Scientific Officer/Principal Scientific Officer grade according to the age, qualifications and experience of the successful applicant.

# SALARY SCALES

S.S.O. £3,157 to £4,441 P.S.O. £4,227 to £5,550

Cost of living supplements are paid in addition to salary.

Non-contributory superannuation scheme.

Application forms and further particulars available from Establishments Division. Approach available from Establishments Division, Natural Environment Research Council, Alhambra House, 27/33 Charing Cross Road, London WC2H 0AX. Please quote reference

Closing date: November 29, 1974.

#### THE HORMEL INSTITUTE OF THE UNIVERSITY OF MINNESOTA

invites applications from qualied individuals for the position of

#### EXECUTIVE DIRECTOR

of the Hormel Institute which is located in Austin, Minnesota. The Executive Director is responsible for overall administration of an institute with 80-90 employees, including 20 of whom have academic rank. Candidates for this position should be recognized authorities in the lipid field, and should have at least 12 years of research experience in the field. The director is expected to maintain an active research program. Closing date for receipt of all materials is February 1, 1975. Send Curriculum vitae, a list of publications and names of three referees to Dr. J. E. Gander (Chairman, Search Committee), Department of Biochemistry, University of Minnesota, St. Paul, MN 55108. (1757)

#### ROYAL COLLEGE OF SURGEONS

Vacancies exist for two technicians in the Pathology Department. Histological experience is necessary for the first; microbiological experience would be an advantage in the second. Starting salary £1,986 p.a. plus Threshold on Whitley scale. Three year tenure.

Please apply Personnel Officer, Institute of Basic Medical Sciences, Royal College of Surgeons of England, 35-43 Lincoln's Inn Fields, London WC2A 3PN, Tel: 01-405 3474. (1759)

# UNIVERSITY OF GUELPH CHAIRMAN OF THE DEPARTMENT OF HUMAN KINETICS

DIRECTOR OF THE SCHOOL OF\*
PHYSICAL EDUCATION

PHYSICAL EDUCATION

Applications are invited for the combined position of Chairman of the Department of Human Kinetics and Director of the School of Physical Education, the appointment to be effective from July 1, 1975, when the present Director will complete his account term of office, or as soon as possible thereafter. The Chairman of the Department of Human Kinetics acts as the Director of the School of Physical Education, as well as administering the affairs of the Department of Athletics through its Director. The Department of Human Kinetics will be entering into the training of graduate students in 1975 as well as carrying a well established undergraduate programme. The position requires a recognized scholar with broad knowledge of human movement. Experience in university teaching is desirable.

movementa. Experience is desirable.

The term of office is three to five years, with a possible extension upon review.

Enquiries and applications, which will be treated in confidence, should be addressed to The Secretary, Selection Committee, Dean's Office, College of Biological Science, University of Guelph, Guelph, Ontario, Canada, NIC 2001 sity of Guelph, Guelph, Ontario, Canada, NIG 2W1.
Closing date for applications is January 1, 1975.

(1765)



# RESEARCH Marine Biology

Southampton Salary c. £4000 - £6000 p.a.

As part of the Board's Biological Research Programme the CENTRAL ELECTRICITY REŠEARCH LABORATORIES maintain a Marine Biological Unit at Fawley on Southampton Water. A biologist is now sought to lead this group, which includes biologists and chemists, in a study of marine and estuarial ecology in relation to electricity generation. The Laboratory is well equipped with experimental and analytical facilities and the successful applicant will have considerable scope for defining the programme. Publication of results is encouraged.

If you are a biologist with a record of scientific achievement and experience in a relevant field we would be interested to hear from you.

Write NOW to the Personnel Officer (Research),  $Central\ Electricity\ Generating\ Board,\ 15\ Newgate$ Street, London EC1A 7AU, quoting Ref. N/354.

> CENTRAL ELECTRICITY RESEARCH LABORATORIES

#### McGILL UNIVERSITY, DEPARTMENT OF BIOLOGY

HUMAN or MAMMALIAN CELL GENETICIST NEUROBIOLOGIST

TERRESTRIAL PLANT ECOLOGIST competent to develop a course in Canadian Flora

VERTEBRATE ETHOLOGIST

Four Assistant Professors will be hired in 1975 to join the regular academic staff in the Biology Department (currently approximately 50 members). Expertise is required in one of the four fields indicated above. The Department has major and honours programmes and groups of staff members conducting research in these four areas. All staff are expected to establish their own research programmes and to take an active part in teaching undergraduate and graduate students.

Applicants should send a curriculum vitae and the names of suitable referees before January 31, 1975 to Ms E. Mader, Administrative Assistant, Department of Biology, McGill University, P.O. Box 6070, Station A, Montreal, Quebec, Canada H3C 3GI. (1813)

# Overseas Development

# Lesotho Agricultural College

# Senior Lecturer— Farm Machinery and Estate Crafts

To plan syllabuses and implement teaching in Farm Machinery, Farm Carpentry, and Welding; plan and supervise workshop equipment; supervise demonstrators in farming crafts; plan and execute programme of practical tests in farming crafts. Applicants should have proven ability in the prescribed duties of the post,

# Farm Director and Senior Lecturer— Farm Management

To plan, direct and manage the College Farm; to draw up long-term management plans for the Farm in the context of the country's agricultural industry for the present and future; design and implement courses in farm management; lecture in elementary farm accounting; records and marketing. Applicants should have a degree or appropriate qualification and be experienced in the prescribed duties of the post.

# Senior Lecturer—Animal Husbandry

To assist in planning and management of livestock enterprises; to teach theory and practical skills with special emphasis on dairy and beef cattle; plan short courses and conferences for the Ministry of Agriculture field staff. Applicants should have a degree or diploma in agriculture and be experienced in the prescribed duties

# Senior Lecturer—Crop Husbandry

Assist in planning and management of grassland and cropping policy; teach theory and practice in these subjects; liaise with the Farm Machinery Lecturer in applica-tion of mechanisation to crop and grassland production; plan short courses for Ministry of Agriculture field staff. Applicants should have a degree or diploma in agriculture and be experienced in the prescribed duties of the post.

Terms (all posts): Salary will be in excess of current U.K. earnings plus a variable tax-free overseas allowance. Appointments for 2 years. Other benefits include free family passages, paid leave, children's education allowances, and free accommodation and medical attention. Superannuation rights may be safeguarded and all emoluments are paid by the British Government. Applicants should normally be citizent of and permantily resident in the United Kingdom. be citizens of, and permanently resident in, the United Kingdom.

For full details and an application form please apply, indicating post concerned, and giving details of age, qualifications and experience to:-

Appointments Officer

Ministry of Overseas Development

Eland House Stag Place London SWIE 5DH

(1845)



#### THE FACULTY OF MEDICINE OF MEMORIAL UNIVERSITY OF NEWFOUNDLAND

will appoint an Endocrinologist to its Basic Sciences Division in 1975. Individuals with research in-terests in metabolic regulation, neuroendocrinology or membrane studies will be considered.

Interested applicants should send resumé and names of three referees to:

Dr. Bruce H. Sells
Laboratories of Molecular Biology
Faculty of Medicine
Memorial University of Newfoundland
St. John's, Newfoundland
Canada

Before December 15, 1974.

(1755)

#### UNIVERSITY OF ALBERTA DEPARTMENT OF ZOOLOGY Edmonton, Alberta T6G 2E1, Canada

Edmonton, Alberta T6G 2E1, Canada
Two positions are available as Assistant or Associate Professor, from July 1, 1975. A specialist in some aspect of invertebrate zoology is needed to take major responsibility for teaching in a general course in invertebrates. A specialist in vertebrate morphology and vertebrate palaeontology; an interest in fishes is preferred. In both positions there will be opportunity for teaching at advanced levels. Strong research orientation and Ph.D., or equivalent, is required. Salary will be within the range Can \$13,440 to 23,416. Applicants send a curriculum vitae and the names of three referees to Dr. J. R. Nursall, Chairman, Department of Zoology, before January 31, 1975. (1756)

#### PENNSYLVANIA STATE UNIVERSITY

DEPARTMENT OF PHYSICS

Assistantships are available for graduate students wishing to pursue a Ph.D. program, starting September 3, 1975. Major research areas: Solid state and surface physics, field ion microscopy, low temperature physics, molecular spectroscopy, laser physics and nonlinear research, acoustics, particle and Q.E.D. theory. Half-time teaching assistantships carry a stipend of \$3.852 for nine months. Quartertime summer teaching appointments and some all-year research appointments are available. Address inquiries to Prof. R. H. Good, Jr., Head, 104 Davey Laboratory, University Park, Pa., USA 16802. (1770)

#### UNIVERSITY OF EAST ANGLIA SENIOR RESEARCH ASSOCIATE

SENIOR RESEARCH ASSOCIATE

Applications are invited from suitably qualified persons for a Senior Research Associateship in the Climatic Research Unit (associated with the School of Environmental Sciences). A good degree in meteorology, geophysics, or geography with acceptable qualifications in meteorology and geophysics, and previous experience of research and practical work on the large-scale circulation of the atmosphere or oceans, are desirable. This post is particularly concerned with a project for mapping the reported weather, and diagnosing the atmospheric circulation patterns prevailing, season by season, year by year, over the past thousand years. Salary on the lecturer scale, from £2.118 to £4.896, starting point determined by age and experience: the possibility exists of appointing a highly qualified person to points on the scale corresponding to a senior lectureship. The appointment will be for 3 years in the first instance, but may be extended for a further period. for a further period.

Applications (one copy only) with a curriculum vitae and list of publications, together with the names and addresses of two referees, to Establishment Officer, University of East Anglia, Norwich NR4 7TJ, England, by December 20, 1974, from whom further particulars may be obtained.

#### UNIVERSITY OF BIRMINGHAM TECHNICIAN GRADE 3

A Research Technician is required to assist in a Neurochemistry laboratory, in the Neurocommunications Research Unit. The work involved would mainly be biochemical with some physiological and animal experimentation. Some laboratory experience essential and preference will be given to applicants with a degree or O.N.C. in Chemistry or Richards subject. Biological subject.

Assistant Secretary, University of Birmingham, P.O. Box 363, Birmingham B15 2TT.

Ref. 581/C/579.

(1763)

#### DEPARTMENT OF ZOOLOGY UNIVERSITY OF CALIFORNIA, **DAVIS**

The Zoology Department has an opening either for an ecologist with research interests in aquatic organisms, or for a behaviorist working on causal mechanisms or genetics of vertebrate behavior. The person appointed will be expected to conduct research and to participate actively in undergraduate and graduate teaching. The appointment will be as an Assistant Professor—salary US \$12,800 to \$15,100. Persons who want to apply should send a curriculum vitae to: The Search Committee, Department of Zoology, University of California, Davis, California 95616, U.S.A.

The University is an Equal Opportunity and Affirmative Action employer. (1767)

#### ABSTRACTOR/TRANSLATOR

A recent graduate in microbiology or allied subject with a good knowledge of French required to prepare abstracts from French research literature in the fields of microbiology and biochemistry. The position is a full time one and part of the time will be spent at one or more of the large London libraries and part at I.R.L.'s offices. Please apply in writing to Dr. E. S. Krudy, Information Retrieval Limited, 1 Falconberg Court, London WIV 5FG. (Quote Ref. T1). (1768)

#### UNIVERSITY OF LIVERPOOL DEPARTMENT OF BIOCHEMISTRY EXPERIMENTAL OFFICER

Applications are invited for the above post to work with a group on some aspects of Affinity Chromatography. Applicants should have research experience and a good honours degree in Chemistry or Biochemistry. The appointment will be made on the scale £1,953 to £2,757 per annum (plus threshold payments) and it is hoped that the successful applicant will take up the post as soon as possible. Application forms may be obtained from the Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote ref RV/301,/N.

#### THE UNIVERSITY OF TEXAS CELL BIOLOGIST

Applications or noninations are invited for the position of Assistant Professor of Zoology. Duties include teaching in undergraduate and graduate courses and developing a strong research program in some area of cukaryote cell biology. Post doctoral research experience helpful. Applicants should submit curriculum vitae, reprints, a short statement of research and teaching plans and the names of three references to: Chairman, Department of Zoology, University of Texas at Austin, Austin, Texas, U.S.A. 78712. The University of Texas is an equal opportunity employer. (1771)

#### UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

AT URBANA-CHAMPAIGN

COMPARATIVE PHYSIOLOGIST, Assistant or Associate Professor, for Fall 1975. Teaching includes comparative physiology of adaptation, metabolism and function of cells and organ systems. Postdoctoral research experience, publications essential. Apply: Dr. John S. Willis, Department of Physiology and Biophysics, University of Illinois, 524 Burrill Hall, Urbana, Illinois 61801. The University of Illinois at Urbana-Champaign is an Affirmative Action/Equal Opportunity Employer and encourages applications from members of minority groups and women. (1772)

#### ACADEMIC OPENING

ACADEMIC OPENING

The Department of Chemistry of Yale University is seeking applications for appointment to our faculty at the Assistant Professor level in inorganic chemistry. The appointment would become effective in the 1975-76 academic year. Expertise in x-ray crystallography is required; however, a research program in structural chemistry should complement a program in another subdiscipline, such as bioinorganic or organometallic chemistry.

Yale has an active Affirmative Action Program and we are especially interested in minority group or female candidates who meet the above criteria.

Candidates should request referees to write and should send a curriculum vitae, a list of publications, brief summaries of predoctoral and any postdoctoral research, and a brief outline of proposed research projects to the Chairman, Department of Chemistry, Yale University, New Haven, Connecticut, 06520. Applications should be sent promptly, preferably by December 31, 1974 to assure consideration.



**HEALTH DISTRICT (TEACHING)** Lambeth, Southwark and Lewisham Area Health Authority (Teaching)

# **Quality Controller**

Applications are infited from pharmacists or graduate chemists for the above post based at King's College Hospital.

The person appointed will be responsible for the management of the Laboratory and accountable to the Area Pharmacist. The work involves setting up procedures for routine analysis. Involvement in Research projects will be encouraged

Salary according to age, experience and qualifications within range £1,872 to £3,120 plus L.W. and Threshold agreements.

From January 1, 1975. £2,325 to £4,377 plus London Weighting. Application forms available from Personnel Department, King's College Hospital, Denmark Hill, London SE5 9RS. Tel: 01-274 6222 Ext. 2724/8

#### AUSTRALIA

MINISTRY FOR CONSERVATION, VICTORIA

# DIRECTOR OF NATIONAL PARKS

National Parks Service, Victoria

Applications are invited for the position of Director of National Parks from persons who have the following qualifications

- A sound knowledge of public or business administration together with broad experience and a proven record as an administrator in a field having a scientific or technical
- A degree in one of the natural sciences. A higher degree
- in the botanical or biological field will be preferred.

  Ability to lead and control staff trained in a variety of disciplines.
- Ability to communicate effectively with staff and with the community.

The Victorian National Parks Service presently controls 24 widely dispersed parks totalling 510,000 acres.

The Service is in a development stage in which new parks are being placed under its control and additional staff are being recruited to strengthen the existing establishment and to create and Interpretative Service Section.

Conditions of Employment-

Salary A \$18,375 per annum. Leave and other conditions equivalent to that applying to the Victorian Public Service. The position is statutory office but line of responsibility and duties may be varied in accordance with pending legislation and recommendations resulting from a Board of Inquiry into the Public Service.

Further particulars may be obtained from, and applications should be submitted to:

Minister for Conservation, 240 Victoria Parade, East Melbourne, 3002, Victoria, Australia.

Applications close on November 29, 1974.

(1830)

# Findyour place in British Gas

## .PHYSICIST

London Research Station carries out research and development work on a wide variety of problems associated with the Gas Industry; the work ranges in scale from pilot-plant chemical engineering and computer control of transmission and distribution networks to the detailed study of elementary chemical and physical processes.

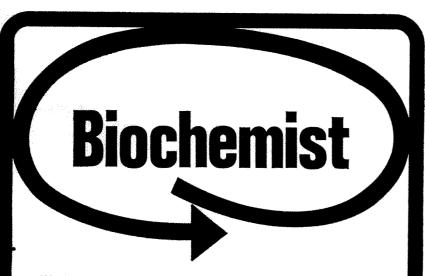
The Physics Division, within the Station, has a vacancy for a Scientist to be responsible for developing and applying a variety of physical measuring devices of potential use to the Gas Industry, e.g. flow measurement, laser, optical and spectroscopic techniques.

Applicants should have a scientific or engineering background to degree standard; experience in the use of any of the devices mentioned above would be an advantage.

Starting salary will depend on qualifications and experience, but will be at a point on scales rising to £3,834 or £4,197 plus Threshold payments.

For further details and an application form, please contact the Research Secretary, British Gas Corporation, London Research Station, Michael Road, Fulham, London, SW6 2AD, quoting reference 4010/14/NA.

**BRITISH GAS** 



We have a vacancy in our Pharmacology Department for a qualified biochemist with either H.N.C. or first degree, Ideally, candidates should have 1-3 years relevant experience but we would consider newly qualified applicants.

The work would involve radio-chemical and chemical analysis of biogenic amines associated with our current research programme in the C.N.S. area.

The laboratories are situated in an attractive 47 acres site and the fringe benefits include, non-contributory pension scheme, B.U.P.A., and four weeks holiday.

Please write giving details of experience to:



The Personnel Manager, Lilly Research Centre Ltd., Erl Wood Manor, WINDLESHAM, Surrey, or telephone for an application form Bagshot 73631. (1849)

# LY RESEARCH CENT

#### University of Bradford LECTURERS (2 Posts) IN THE SCHOOLS OF CIVIL AND STRUCTURAL ENGINEERING

Applications are invited for the above posts. For one post preference will be given to applicants with good re-inforced concrete design experience.

Salary within scale £2,118 to £4,896 p.a. Superannuable

Further particulars and application forms Further particulars and application forms (to be returned a.s.p.) obtainable from the Registrar, Posts CE/L/12 and 17, University of Bradford, Richmond Road, Bradford BD7 1DP, West Yorkshire, Informal enquiries to Professor C. B. Wilby. (1803)

#### M.R.C. CLINICAL RESEARCH CENTRE.

(Northwick Park Hospital), Watford Road, Harrow, Middlesex, HA1 3UJ.

Harrow, Middlesex, HA1 3UJ.

TECHNICIAN for work on the chemical study of some inborn errors of metabolism. The programme is particularly concerned with inherited metabolic diseases in which there appears to be a primary abnormality of organic acid metabolism and it would be suitable for a holder of H.N.C. in chemistry with experience in gas chromatography. Ref. 123D/2B/4222.

Salary within the range £1.986 to £2.961 plus Threshold increase.

Further details and application forms from Mrs J. Tucker-Bull. Please quote reference. (1775)

#### M.R.C. CLINICAL RESEARCH CENTRE,

(Northwick Park Hospital). Watford Road. Harrow, Middlesex, HA1 3UJ.

JUNIOR TECHNICAL OFFICER/TECHNICAL OFFICER to work in the Division of Psychiatry on biochemical and physiological investigations in psychiatric illness. Experience in neuropharmacological techniques an advantage. Candidates must hold H.N.C. or ordinary degree in relevant subject.

in relevant subject. Salary within the range £1,986 to £2,961 plus Threshold increase, depending upon qualifications and experience.

Ref. 125/2/A30.

Please apply to Mrs J. Tucker-Bull for application form and further details. (1776)

#### UNIVERSITY OF ABERDEEN SENIOR LECTURESHIP/ LECTURESHIP IN GEOLOGY AND MINERALOGY PETROLEUM EXPLORATION STUDIES

Applications are invited for the above posts (two vacancies). Preference will be given to candidates with special interests or experience in any of the following fields: Petroleum Geology, Well Logging, Formation Evaluation, Reservoir Studies, Salary on scale: Senior Lectureship £4,707 to £5,844 per annum. Lectureship £2,118 to £4,896 per annum with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (3 copies) should be lodged by December [4, 1974. (1764)

#### VICTORIA UNIVERSITY OF WELLINGTON NEW ZEALAND

#### SENIOR LECTURER OR LECTURER IN PHYSICAL CHEMISTRY

IN PHYSICAL CHEMISTRY

Applications are invited for the above-mentioned post; it is hoped that the successful applicant will have interests related to the fields of research at present being pursued in the Department, in particular transport properties, structure, reaction kinetics and electrochemistry of the liquid and solid states including mineral systems, especially pressure and temperature effects.

Salary range: Lecturer NZ\$7,361 to \$9,339 p.a. Senior Lecturer NZ\$9,503 to \$11,153 bar \$11,484 to \$12,142. The level of appointment and the initial salary wil be determined by the qualifications and experience of the appointee. An allowance is made towards travel and removal expenses.

Further particulars and application procedure available from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H OPF.

Applications close November 30, 1974. (1779)

Applications close November 30, 1974, (1779)

#### UNIVERSITY OF BRISTOL DEPARTMENT OF SURGERY

Applications are invited for the post of RESEARCH ASSISTANT/ASSOCIATE

to collaborate in a study involving the adoptive transfer of immunity to patients with advanced malignant disease.

Candidates must posses a Ph.D.

The post is available for two years, in the first stance, from March 1, 1975, and carries a salary up to £2,412 per annum (plus F.S.S.U.) according to experience

Applications in writing with the names of two referees to Professor J. H. Peacock, Department of Surgery, Bristol Royal Infigurary, Bristol B28HW as soon as possible please. (1777)

#### THE ROYAL HORTICULTURAL SOCIETY

#### APPOINTMENT OF BOTANIST

Applications are invited for the post of Botanist at the Society's Garden at Wisley.

The duties will include advisory work on plant identification, and physiology, genetics and chemistry related to horticulture; demonstrations to trainees, cataloguing and labelling plants at Wisley and Committee work. Graduates in botany or horticulture preferred.

The salary, reviewed annually, will be related qualifications and experience. The post is pensionable.

Applications with details of qualifications and experience and the names of two persons to whom reference may be made should be sent so as to reach the Secretary, Royal Horticultural Society, Vincent Square, London SWIP 2PE, by Monday December 2, 1974. (1783)

#### UNIVERSITY OF BRISTOL DEPARTMENT OF BIOCHEMISTRY POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited from candidates with re-Applications are invited from candidates with research experience in protein chemistry and/or enzymology for a Postdoctoral Research Assistantship to work in collaboration with Dr J. Williams on an M.R.C. supported project involving a structural and functional investigation of metal-binding fragments from transferrin.

The appointment is available now for a period of up to three years at an initial salary up to £2,412 (plus benefit of Threshold Agreement supplement as appropriate).

Interested candidates should write to: Dr J. Williams, Molecular Enzymology Laboratory, Department of Biochemistry, University of Bristol, Medical School, Bristol BSS 1TD. (1784)

#### UNIVERSITY OF BRISTOL DEPARTMENT OF BOTANY **TECHNICIAN GRADE 5**

There is a vacancy for an Electron Microscopist Grade 5 in the Department of Botany. The person appointed would have to accept overall responsibility for the operation, full maintenance and servicing of three microscopes A.E.I. E.M. 6G. A.E.I. E.M. 6B. and Cambridge Instrument Stereoscan S.4. together with ancillary equipment. Familiarisation courses could be arranged for otherwise suitable candidate. Salary £2,007 per annum on scale to £2,382 per annum. Threshold supplements are also payable under current arrangements. (Salary scale under review). (Salary scale under review).

(Salary scale under review).

Applications, giving full details of qualifications and experience, together with names of two referees, should be sent to the Laboratory Superintendent, Department of Botany, University of Bristol, Woodland Road, Bristol BSS 1UG. (1785)

#### MEMORIAL UNIVERSITY OF NEWFOUNDLAND **CANADA**

DEPARTMENT OF BIOLOGY Applications are invited for the position of BACTERIAL SYSTEMATIST

BACIERIAL SISIEMAINI

The successful candidate must also have a good background in either Bacterial Physiology or Immunology. A background in Bacterial Genetics would also be desirable. Appointment will be made at the Assistant Professor level, to commence September 1, 1975. Applicants should have a strong interest in undergraduate teaching and be capable of initiating an independent research programme.

Closing date for receipt of applications: March 15, 1975. Apply to Professor J. Phipps, Head. Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada. (1788)

# **AUSTRALIA** THE AUSTRALIAN MUSEUM, SYDNEY, NEW SOUTH WALES

# **CURATOR/ASSISTANT CURATOR**

(two positions)

The Australian Museum is located in Sydney and has a total staff of over 140 with 12 departments covering all systematic groups of animals plus Environmental Studies, Palaeontology, Mineralogy and Anthropology. The scientific staff numbers 30. Field research is emphasised and a wide range of field equipment is available. Applications are invited for the above positions.

Salary:

Assistant Curator \$A7,287 to \$A11,322 per annum; Curator \$A11,862 to \$A12,619 per annum; with progression to \$A13,526 per annum subject to certain condition.

# **CURATOR/ASSISTANT CURATOR**

#### DEPARTMENT OF MOLLUSCS

Qualifications:

Essential: Degree with major in Zoology.

Desirable: Postgeaduate qualifications with research in Zoology. Preference will be given to candidates with experience in terrestrial and/or fresh water molfuscan taxonomy and biology. Previous experience in management of museum collection will be an advantage.

**Duties:** 

Research on taxonomy/biology of Mollusca; curation of collections of Mollusca; answering public and professional scientific enquiries.

# **CURATOR/ASSISTANT CURATOR**

### **DEPARTMENT OF ENVIRONMENTAL STUDIES**

**Oualifications:** 

Essential: A degree in some aspect of Marine Ecology, demonstrated ability to initiate and conduct research.

Desirable: Experience in planning and supervision of team research. Preference will be given to an applicant with research interests in estuarine ecology and man/environment problems.

**Duties:** 

Initiate and conduct ecological research and environmental surveys in the marine environment; answer public and professional scientific enquiries relating to marine ecology and human impact on the marine environment. Subject to certain conditions the successful applicants will be eligible for:-

Payment of fares to Sydney;

Financial assistance towards cost of removal expenses;

Financial assistance towards initial accommodation expenses.

For further information and application form telephone or write to the Recruitment Section, New South Wales Government Offices, 66 Strand, London WC2N 5LZ (Tel: 01-839 6651 Extension 194), where applications close on MONDAY, DECEMBER 2, 1974. When telephoning or writing, please quote reference 44/576 (N).

#### DIRECTOR

Applications are invited for the position of DIREKTOR DES INSTITUTS FÜR TIERZUCHT UND TIERVERHALTEN FORSCHUNGSANSTALT FÜR LANDWIRTSCHAFT,

a federal research institution engaged in reproductive biology, animal behaviour and genetics. Staff of 250 including 16 scientists. Research budgeted Facilities: 2,500 m² of laboratory space; 4 research farms (1200 ha): live stock of 1,200 cattle. 2,000 pigs. 1,000 sheep. Locations: main laboratories and 2 farms at Mariensee near Hannover; laboratory and 2 farms at Trenthorst near Lübeck.

Qualifications: Internationally established research reputation. Applicants with biochemical and physiological background should be enabled to coordinate the research programmes of the different

coordinate the research programmes of the different

sections.
Salary: approx. 65,000 DM/annum (B 3 tenure position in government service).

position in government service).

To apply, send curriculum vitae, summary of research activities, list of publications and reprints before December 31, 1975 to:

Prof. Dr E. Zimmer
Präsident of
Forschungsanstalt für Landwirtschaft
Bundesallee 50
D 33 Braunschweig
Federal Republic of Germany

(1792)

(1792)

# **Pharmacist**

required for research and developlaboratories. Applicants must have experience in research, development and testing of new pharmacoutical dosage forms. Salary commensurate with age and experience. Application forms from: The Secretary, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London N1 2HB. (1801)

# LECTURER IN **HUMAN PHYSIOLOGY**

Applications are invited for the above post in the Department of Biological Sciences from those holding an Honours degree in an appropriate discipline. Preference will be given to candidates with a higher degree and with experience in advanced teaching and research. The duties of the post will be largely associated with a recently established Honours degree course in Applied Biology.

Salary Scale: Up to £4,017 per annum depending upon experience, plus £123 per annum for approved teacher training. Application forms and further particulars may be obtained

> The Director, Glasgow College of Technology, North Hanover Place. Glasgow G4 0BA,

and should be returned within ten days of the appearance of this advertisement (1835)

#### NORTH LONDON **BLOOD TRANSFUSION CENTRE** TISSUE TYPING

Technician/Scientific Officer required to assist in antibody screening and reagent preparations. Previous experience in this field would be advantageous but is not essential.

#### MICROBIOLOGY

Scientific Officer required to work on development of techniques for detection and assay of Tetanus antibody in human sera. Experience in serology or biochemistry desirable.

#### **BLOOD GROUP SEROLOGY**

Scientific Officer required for quality control section. Experience in blood group serology essential and automated techniques desirable.

Salaries and conditions are those of the appropriate Whitley Council. Applications and enquiries to the Director. Deansbrook Road, Edgware, Middx. HA8 9BD.

iddx. HA8 9BD. Closing date 2 weeks after the date of insert. (1786)

# university of wales universit

#### RESEARCH ASSISTANT

Applications are invited for the vacancy of Research Assistant in the Department of Genetics to work on assay of genetic damage by heavy metals in marine organisms. Applicants should be graduates in a biological subject with research experience preferably in genetics or cytogenetics.

The appointment, which will commence as soon as possible, will be for one year in the first instance, and will be in the range £1,953 to £2,058 per annum.

Further particulars and application forms may be obtained from the Registrar/Secretary, University College of Swansea, Singleton Park, Swansea SA2 8PP to whom they should be returned by Tuesday, November 19, 1974.

DEPARTMENT OF GEOLOGY

#### UNIVERSITY OF WINDSOR RESEARCH ASSOCIATE

required to fill a permanent position. Applicants should have a Ph.D. or M.Sc. in geoscience. Research studies will be major and minor element geochemistry and/or into the physical properties of rocks and glacial deposists. Teaching duies will be minor. Salary will be commensurate with qualifications. Send résumé with names of three potential referees to:

Dr D. T. A. Symons
Department of Geology
University of Windsor
Windsor, Ontario, Canada N9B 3P4

(1791)

Applications are invited for (a) SENIOR LECTURESHIP or (b) LECTURESHIP IN CLINICAL PHYSIOLOGY, tenable as soon as possible. Applicants should possess a medical qualification and relevant postgraduate training. Duties include lecturing in basic and clinical Physiology and demonstrating in experimental human and animal physiology to students working for the medical degree.

PURDUE UNIVERSITY

Postdoctoral Research determining the structures of polynucleotides and polysaccharides mainly by X-ray fiber diffraction analyses wanted for 1-3 years. Send curriculum vitae with names of two referees to:

Professor Struther Arnott Department of Biological Sciences Purdue University West Lafayette, Indiana 47907

#### UNIVERSITY OF THE WEST INDIES **JAMAICA**

degree.

Salary scales: (a) J\$10,992 to J\$14,472, (b) J\$7.860 to J\$10,752 p.a. (£1 sterling=J\$2.12) Additional renumeration will be available for all clinical duties. F.S.S.U. Unfurnished accommodation for a maximum of three years at 10% of salary. Therafter 20% of salary payable in lieu of housing. Family passages; triennial study leave. Detailed applications (3 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail as soon as possible to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica. Detailed particulars are available and should be obtained from the same source before an application is made. (1787)

#### NATIONAL COUNCIL FOR SCIENTIFIC RESEARCH

Applications are invited from suitably qualified persons for the post of

#### TIMBER RESEARCH OFFICER

Applicants must have a degree or equivalent pro-fessional qualification in timber or structural engineering and experience in the use of timber in the mining and other industries.

The timber research officer's duties will include making surveys of timber and forest products utilization by the Zambian industries including the mining industry, planning and executing applied research in the use of timber and forest products and liaising with industries on timber and forest products utilization.

Salary according to qualifications and experience on the scales:— Senior Professional Officer K4,680 by 240 to K5,640

Principal Professional Officer
K5,840 by 240 to K6,800
Senior Principal Professional Officer
K7,000 by 200 to K7,600

K7,000 by 200 to K7,600

There is a superannuation scheme for Zambians. Non-Zambians will be paid a graduated gratuity of 20% of the total earnings in the first year. 25% in the second year and 30% in the third year of resident service. Passages will be paid for the officer, wife and minor dependent children.

Applications giving full personal details, qualifications, experience and naming three referees should be submitted to:

The Secretary General,
National Council for Scientific Research,
P.O. Box CH 158,
Chelston, Lusaka,
Zambia. (1794)

#### IMPERIAL COLLEGE NON-STOICHIOMETRIC OXIDE ELECTRODES

Applications are invited for a three year postdoctoral research assistantship. The project is principally concerned with the characterisation of nonstoichiometric oxides using high resolution electron
microscopy, and subsequent correlation of the defect
structure with electromechanical behaviour. Excellent
facilities are also available for the examination of
oxide materials in the 1 mev electron microscope.
Starting salary in the range £2.118 to £2.412 plus
London allowance, threshold payments, and
F.S.S.U. benefits. Applications should be made to
Dr B. C. H. Steele, Department of Metallurgy and
Materials Science, Imperial College, London
SW7 2BP.

#### STAFFORDSHIRE AREA HEALTH AUTHORITY SOUTH EAST STAFFORDSHIRE HEALTH DISTRICT THE GENERAL HOSPITAL

BIOCHEMIST (Basic Grade)

required to work under the Principal Biochemist in well equipped laboratory at the above hospital.

Single accommodation may be available.

Further information from the Principal Biochemist, The General Hospital, Burton-on-Trent. Applications stating experience and naming two referees to Personnel Officer, Burton District Hospital Centre, Belvedere Road, Burton-on-Trent DE13 0RB. (1799)

#### COMMONWEALTH AGRICULTURAL **BUREAUX**

VACANCY FOR

SCIENTIFIC INFORMATION OFFICER AT THE

#### COMMONWEALTH FORESTRY **BUREAU**

COMMONWEALTH FORESTRY INSTITUTE. SOUTH PARKS ROAD, OXFORD OX1 3RD

**Duties:** Abstracting and indexing of technical papers and dealing with enquiries.

Qualifications: Applicants should have a reading knowledge of some foreign language(s), and should be graduates in forestry or biology, or linguists with an interest in biology. Opportunities for further training.

training.

Salary: In scale £1,592 to £2,996 (bar), £3,157 to £3,943 (bar), £4,275 to £4,505, plus a compensatory allowance (taxable but not superannuable) of 4½% to offset personal contribution to F.S.S.U., and threshold payments to date. Starting salary, according to qualifications, age and experience, will be in the lower sector of the scale.

Application forms and full particulars from the

Application forms and full particulars from the Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN. Closing date for applications: November 30.

(1791)

(1789)

#### TWO VISITING INSTRUCTORS OR ASSISTANT PROFESSORS OF BIOLOGY

ASSISTMENT PROFESSORS OF BIOLOGY
to be appointed for two years each. Teaching duties
include: Introductory Biology and two of the following—Embryology, Cell Physiology, Vertebrate Histology and General Ecology, Interested person
should send curriculum vitae, transcripts and three
letters of reference by January 10, 1975 to: Chairman Search Committee, Biology Department, Kenyon College, Gambier, Ohio 43022.

Kenyon College is an equal-opportunity employer
(1802)

#### UNIVERSITY OF MELBOURNE CHAIR OF PSYCHOLOGY

CHAIR OF PSYCHOLOGY

Applications are invited for the Chair of Psychology which has become vacant following the resignation of Professor A. Heron, It is hoped that the successful applicant will take up duties on August 1, 1975 or as soon as possible thereafter.

Salary: \$A19,614 per annum (under review). Further information, including details of application procedure, superannuation, travel and removal expenses, housing assistance and conditions of appointment, is available from the Association of Commonwealth Universities (Appts). 36 Gordon Square, London WC1H 0PF.

Applications close on January 10, 1975.

(1806)

#### MONASH UNIVERSITY Melbourne, Australia DEPARTMENT OF BOTANY SENIOR TUTOR

SENIOR TUTOR

Applications are invited for the post of Senior Tutor in the Department of Botany, from those with research interests in any area of the subject. Candidates will be expected to have gained a Ph.D. or have equivalent experience. The successful applicant will be expected to earry out research in the field of his specialty, to help with the planning and running of first-year laboratory classes, and to do some lecturing and demonstrating in later years.

Salary Range: \$A7,545 to \$A9,002 per annum with superannuation based on an endowment assurance scheme, the employee and employer contributing 5% and 10% respectively.

Benefits: Travelling expenses for appointee and family; removal allowance; temporary housing for an initial period, repatriation after three years service if desired.

Further general information and details of application procedure are available from the Academic Registrar, Monash University, Wellington Road. Clayton, Victoria 3168, Australia or the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WCIH 0PF. Enquiries about the Department to the Chairman, Professor M. J. Canny, in the University, Applicants should quote reference No. 43013.

Closing Date: November 25, 1974.

The University reserves the right to make no appointment or to appoint by invitation.

#### ROYAL POSTGRADUATE MEDICAL SCHOOL POSTDOCTORAL BIOCHEMIST

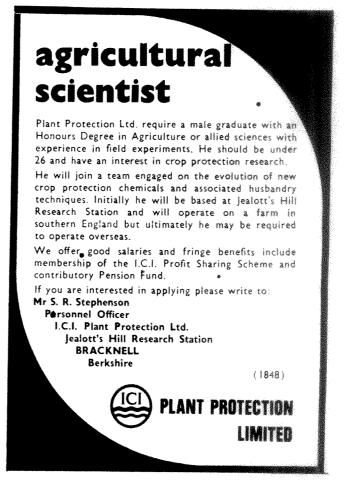
required to study the enzymology of carboxylases both from animal tissues and children with inborn errors of metabolism affecting mitochondrial carboxylases. This position is available for 1 year in the first instance with the possibility of extension for a further two years. Starting salary up to £2.511 per annum according to experience.

Applications including full curriculum vitae to the Personnel Officer, R.P.M.S. Hammersmith Hospital, Du Cane Road, London W12 0HS, quoting Ref. No. 2/474N. ((1812)

#### UNIVERSITY OF MANCHESTER SENIOR LECTURER IN EXPERIMENTAL PATHOLOGY

EXPERIMENTAL PATHOLOGY

Applications are invited for this post in the Department of Pathology. The post involves teaching the principles of Pathology and insearch within a group investigating the immunological and migratory properties of lymphocytes. This group which is partly supported by an M.R.C. programme is concerned with the recognition structures on lymphocytes and endothelial cells which govern the migration of lymphocytes from the blood into the tissues. For this project experience of membrane fractionation techniques or glycoprotein chemistry would be relevant. A second project is concerned with the recognition of transplantation and other antigens by T cells. The post will not carry clinical duties. Salary range p.a.: £4,707 to £5,844. F.S.S.U. Further particulars and application forms (returnable by December 21) from the Registrar, The University, Manchester M13 9PL. Quote Ref. 229/74/N. (1809)



#### WANTED

A postdoc in population biology interested in genetic structure of populations and species to con-duct research 1975-6, on a grant basis. Send information, curriculum vitae, references

and reprints to:
Prof. Eviatar Nevo.
Department of Biology,
University of Haifa,
Israel.

(1800)

#### UNIVERSITY OF OXFORD DEPARTMENT OF BIOCHEMISTRY

A Postdoctoral Research Assistant is required for work on the structures, kinetics and mechanisms of pyridine-nucleotide linked dehydrogenases, financed by the S.R.C. and beginning as soon as possible. The appointment will be for one year in the first instance, at a starting salary of not less than £2,010 p.a., plus threshold payment (at present £166 p.a.), with a possibility of further extension.

extension.

Applications, including curriculum vitae and the names of two referees, should be sent to:
Dr K. Dalziel,
Department of Biochemistry,
University of Oxford,
South Parks Road,
Oxford OX1 3QU.

(1816)

### THE POLYTECHNIC WOLVERHAMPTON

WELLCOME TRUST RESEARCH ASSISTANT

Applications are invited for a research assistant to participate in a project (supported by the Wellcome Trust) to investigate the function of intestinal peroxisomes with special reference to a role in lipid and cholesterol metabolism. The project will be supervised by Dr. M. Connock and the post is for three years. Applicants should hold, or be about to receive, a Ph.D. in Biochemistry, Physiology or related subject.

Commencing salary is up to £2,223 per annum. Further details can be obtained from The Establishment Officer, The Polytechnic, Wolverhampton WV1 1LY.

#### LIVERPOOL SCHOOL OF TROPICAL MEDICINE

DEPARTMENT OF PARASITOLOGY

DEPARTMENT OF PARASTOLOGY

A LECTURER/SENIOR LECTURER (Technical Assistance) is required in this Department, Applications are invited from candidates holding a medical qualification registrable in the U.K. of a good science degree and doctorate in relevant subjects. Practical experience in the parasitology of tropical diseases is essential and preference will be given to candidates who already have experience in teaching and/or research on parastic diseases in the developing countries.

The successful applicant will be required to spend periods of service overseas at teaching and/or research centres but will be based in this Department.

ment.
Salary will be within the University scale for Lecturer/Senior Lecturer and the starting point will be commensurate with qualifications and experience. The appointee will be entitled to participate in the University Pension Scheme, Further information may be obtained from the understance.

Further information may be obtained from the undersigned.

Applications together with the names of three referees should be sent to the Dean, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, to arrive not later than December 15, 1974. Overseas candidates should apply by airmail.

PHARMACOLOGIST, experienced, medical or veterinary qualifications preferred. required for pharmacological research and toxicological studies of compounds of potential therapeutic importance. Excellent apportunities for advancement in modern. well-equipped laboratory, Pension Assurance Scheme. Application forms from: The Secretary, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London (1814)

#### UNIVERSITY OF STRATHCLYDE DEPARTMENT OF PURE & APPLIED CHEMISTRY

**TECHNICIAN GRADE 3** 

A vacancy exists for an experienced Technician qualified to at least O.N.C. in Chemistry, or a graduate, to assist with a project in Organometallic Chemistry.

This post is for a period of three years.

Salary Scale: £1,650 to £1,920 plus threshold payment

payment

Applications in writing, quoting reference C.81, should be made to The Personnel Officer, University of Strathclyde, Royal College Building, 20 George Street, Glasgow G1 1XW. (1819)

#### UNIVERSITY OF WARWICK RESEARCH TECHNICIAN

The Department of Biological Sciences has a vacancy in the microbiology research laboratories for a Research Technician. Applications are invited from persons with experience of biochemical and/or microbiological techniques. The post is for a fixed contract period terminating on September 30, 1975. Salary on the Technician Grade 3 scale £1,650 by £54 to £1,920 p.a. plus threshold payments.

Applications in writing quoting Ref. No: 13/D/74 to the Academic Registrar, University of Warwick, Coventry CV4 7AL by November 18, 1974.

#### LONDON SCHOOL OF HYGIENE AND TROPICAL **MEDICINE**

(University of London) Keppel Street, WC1E 7HT

ROSS INSTITUTE OF TROPICAL HYGIENE SENIOR LABORATORY

# **TECHNICIAN**

Applications are invited from suitably qualified and widely experienced persons in medical laboratory subjects for a post as Senior Technician in the Department. Present research work includes immunofluorescent diagnosis, parasitology, insect genetics and experimental pathology: an interest in tropical medicine an advantage. Candidates should hold H.N.C./F.I.M.L.T. or be graduates in a relevant discipline. Salary, depending on age, qualifications and experience up to £3,573 plus £126 London Weighting and threshold payments. Possibilities of promotion to higher grades. Pensionable post; interest-free season ticket scheme. Applications in writing, giving full career details and naming two referees, to Secretary (AI) by December 16, 1974. Preliminary inquiries to Professor D. Bradley (01-636 8638 ext. 210).

#### TWO TECHNICIANS (Qualfied)

required for research in the Department of Medicine. One post involves the use of immunological and tissue-culture techniques, the other the use of radioimmunoassays. Previous experience preferable but not essential. Apply in writing, stating age and giving details of education, qualifications and experience, to the Secretary, Guy's Hospital Medical School, London Bridge, SEI 9RT, quoting Ref. D.M. (1824)

#### **BIOCHEMIST**

Investigation field: Calcium metabolism. Metabolism of P.T.H. and Calcitonin. Immunradiometric assays for different hormone species available. Radioreceptor assay for human P.T.H. Optimal financial and research conditions. Dr. R. D. Hesch, 34 Goettingen, Med. Univ. Klinik, Endocrinology, Germany. (1829)

#### UNIVERSITY OF EDINBURGH DEPARTMENT OF BOTANY RESEARCH ASSOCIATE IN PLANT TAXONOMY

Applications are invited from Honours graduates with postgraduate experience for the post of Research Associate financed by the Science Research Council to collaborate in writing the Flora of Turkey.

The salary range is £2,118 to £2,757 per annum, with F.S.S.U., depending on qualifications and taxonomic experience.

Further details may be obtained from Dr. P. H. Davis, Department of Botany, Royal Botanic Garden, Edinburgh EH3 5LR. The closing date for applications is November 23, 1974. Please quote reference 5053. (1815)

The Department of Physics at the Instituto Venezolano de Investigaciones Científicas (I.V.I.C.) has openings for experimental physicists with strong research curriculum in neutron physics, low temperature physics, quantum optics or magnetic resonance (E.S.R. and N.M.R.). Participation in graduate teaching is also desired. Preference will be given to Spanish speaking applicants. The positions can become permanent after one year. The salaries depend on experience but are typically around \$1,500.—Applications (including recommendations) should be submitted to: George Bemski, Centro de Fisica, Instituto Venezolano de Investigaciones Científicas, Apartado 1827. Caracas 101. Venezuela. (1826)

#### SEALE-HAYNE COLLEGE LECTURER IN THE DEPARTMENT OF PHYSICAL SCIENCES

Applications are invited from suitably qualified AGRICULTURISTS OR CHEMISTS. Candidates should have interests in one or more of the following:—

# ANIMAL NUTRITION SOIL SCIENCE FOOD SCIENCE

The main courses taught at the College include five Higher National Diplomas, two Ordinary National Diplomas as well as post-graduate/post-diploma certificate courses.

The appointment will be at either the Lecturer Grade I or Lecturer Grade II, Scales of Salaries for Teachers in Further Education, according to age and experience. Further particulars may be obtained from The Principal, Seale-Hayne College, Newton Abbot, Devon, to whom applications should be sent as soon as possible.

#### UNIVERSITY OF VICTORIA DEPARTMENT OF CHEMISTRY FACULTY POSITION IN INORGANIC CHEMISTRY

Applications are invited for a faculty position (professor or senior associate) in Inorganic Chemistry to commence July 1, 1975. Applications will also be considered from outstanding candidates at a more junior level.

The appointee will be expected to participate fully in departmental undergraduate and graduate teaching and in research.

Applicants should submit their curriculum vitae, including biographical information, a list of publications, and a statement of research interests and should arrange at the time of application for three letters of recommendation to be forwarded

Dr. A. D. Kirk, Chairman, Department of Chemistry, University of Victoria, Victoria, B.C., Canada V8W 2Y2.

(1827)

#### IMPERIAL COLLEGE UNIVERSITY OF LONDON LECTURESHIP IN PETROLEUM GEOLOGY

Applications are invited for a lectureship in the department of geology under the direction of Professor W. D. Gill.

Requirements are a good degree and practical experience in the petroleum industry.

Salary depending on qualifications and experience in the scale £2,118 to £4,896 plus £213 London allowance. Superannuation under F.S.S.U. scheme, Applications with curriculum vitae and names of two referees to:

two referees to:

Professor W. D. Gill,

Department of Geology,

Imperial College of Science and Technology,

London, SW7 28P.

Closing date November 30, 1974. (1.

#### DEPARTMENTAL ASSISTANTS

DEPARTMENTAL ASSISTANTS

One or two vacancies for organic chemists of postdoctoral status. Appointment: 2 years, extension to 3 possible. Duties: collaboration with one of the research groups (for one appointment preference will be given to candidates interested in peptide synthesis) and supervision of undergraduate experiments. Salary: from £2,118 plus threshold supplements and superannuation (at present F.S.U. but it is likely that other arrangements will be introduced before October 1975). A successful candidate would be allowed to undertake some college teaching in addition.

Starting date: October 1, 1975. Applications with the names of two referees to the Administrator, Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY as soon as possible before January 31.

### UNIVERSITY OF **CAMBRIDGE**

DEPARTMENT OF ZOOLOGY

A technician is urgently required to assist in the Biological Microprobe Laboratory, which is concerned with the X-ray microanalysis of ions and electrolytes in frozen hydrated biological tissues.

O.N.C./H.N.C. or equivalent qualification required with 3 to 4 years' experience. Some training and an interest in electronics is essential, and general workshop and laboratory experience would be an advantage. Training and experience in biology and transmission electron microscopy is not required.

transmission electron microscopy is not required.

The salary range is £1,650 to £1,920 plus threshold payments.

Applications and enquiries should be addressed to The Secretary, Department of Zoology, Downing Street, Cambridge CB2 3EJ, (Cambridge 58717).

Closing date: November 30, 1974.

(1818)

#### FLINDERS UNIVERSITY OF SOUTH AUSTRALIA

SCHOOL OF MEDICINE

#### SENIOR LECTURER/LECTURER IN CLINICAL PHARMACOLOGY

Applications are invited for a Senior Lecture-ship/Lectureship in Clinical Pharmacology in the new School of Medicine.

new School of Medicine.

The School of Medicine will form an integral part of the Flinders Medical Centre, a 700-bed teaching hospital located on campus at Bedford Park, Adelaide. The first medical students began in 1974 and it is hoped that the appointee will take up his position by mid-1975 in order to participate in the teaching of pharmacology in the early years of the integrated curiculum.

A suitable position propositor will have re-

A suitably qualified appointee will have responsibility in the areas of teaching, research and clinical service. Lecturers with medical qualifications registrable in South Australia receive a clinical loading, the amount depending on their qualifications and the degree of clinical responsibility undertaken.

Conditions of service include provision for transportation expenses, superannuation, tenure, study and long service leave.

Present salary scales are Lecturer—\$A9,002 to \$A12,352; Senior Lecturer—\$A12,643 to \$A14,724. Superannution is on the F.S.S.U. basis.
Further information may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WCIH 0PF.

Applications should be lodged with the Registrar, The Flinders University of South Australia, Bedford Park, South Australia 5042, in duplicate, by November 29, 1974.

#### UNIVERSITY OF EXETER DEPARTMENT OF PHYSICS

#### POSTDOCTORAL RESEARCH **ASSISTANTSHIP**

Applications are invited for a Postdoctoral Research Assistantship in Theoretical Physics supported by the Science Research Council. The research work will involve the use of N-body Faddeev methods to study molecular rearrangement processes at thermal energies; the techniques to be used could be of interest to applicants with experience in either Atomic, Molecular, High-Energy or Nuclear Physics.

Atomic, Molecular, High-Energy or Nuclear Physics.
The appointment is for two years (initial salary £2,118 plus threshold payments plus superannuation benefits) at a starting date to be agreed but preferably not later than April 1, 1975.
Informal enquiries may be addressed to Dr. T. W. Preist, Department of Physics, University of Exeter, Stocker Road, Exeter EX4 4QL, (Tel. No: Exeter 77911, Ext. 452).
Candidates should forward four copies (overseas candidates 1 copy) of their application giving details of their qualifications and experience together with the names and addresses of two referees to The Secretary of the University, Northcote House, The Queen's Drive, Exeter EX4 4QJ not later than December 2, 1974. Please quote reference 12/2/7079 in all correspondence.

#### UNIVERSITY OF SYDNEY LECTURESHIPS AND SENIOR LECTURESHIPS IN BEHAVIOURAL AND SOCIAL SCIENCES

Applications are invited from candidates with postgraduate training in one or more of the behavioural sciences especially from those with some training and/or experience in the application of their discipline in the health and medical fields. The appointees will be involved in the teaching and further planning of a new course in the behavioural and social sciences designed for medical undergraduates. The first year of the course was begun in 1974.

Salary range: Lecturer—\$A9,002 to \$A12,352 p.a.; Senior Lecturer—\$A12,648 to \$A14,724 p.a. The positions advertised are permanent ones, but may be filled for three years in the first instance, with the possibility of permanent after that time.

that time.

Applications, including curriculum vitae, list of publications and names of referees, by December 11, 1974, to the Registrar, University of Sydney, N.S.W. 2006, Australia. Further information can be obtained from the Registrar or from the Acting Head, Department of Behavioural Science in Medicine: Dr W. L. Walker.

Conditions of appointment and application procedure from Association of Commonwealth Universities (Appts), 36 Gordon Square, London WCIH 0PF.

(1837)

### M.R.C SCIENCE GRADUATE

SCIENCE GRADUATE
FOR BIOCHEMISTRY DIVISION
The National Institute for Medical Research has a vacancy for the post of JUNIOR TECHNICAL OFFICER in the biochemistry division. The research being carried out is concerned with the preparation and characterisation of mammalian cell membranes. As a theoretical understanding of the research would be useful, applicants should preferably have graduated in biochemistry or have studied a biological subsidiary. Salary scale starts from £1,875 p.a. plus £167 p.a. threshold agreement (maximum £2,718 plus £167 p.a.). To obtain application forms please write quoting ref. JTO/BM to Mrs M. Y. Jim, Assistant Personnel Officer, National Institute for Medical Research, The Ridgeway, Mill Hill NW7 1AA, Tel: 01-959 3666. (1842)

#### UNIVERSITY OF LONDON CHAIR OF ANATOMY AT KING'S COLLEGE

Re Advertisement—Extension of Closing date. The Senate invite applications for the above Chair. Candidates should have high scientific ability in Anatomy or related fields and a strong interest in the application of their subject to man in the context of Medical Education, Applicants need not necessarily be medically qualified. Initial salary to be aggreed but will not be less than £6,105 per annum plus £213 London Allowance. Applications (11 copies) should be received not later than February 3, 1975, by the Academic Registrar, (N) Senate House, Malet Street, London WC1E 7HU, from whom further particulars concerning the post and its special relationship with medical and biological teaching within the KTW Biomedical Centre and School of Biological Sciences, may be obtained. (1850) Re Advertisement-Extension of Closing date.

#### FELLOWSHIPS AND **STUDENTSHIPS**

#### POSTDOCTORAL FELLOW OR RESEARCH ASSOCIATE

Available immediately for research into laboratory culture and studies of larval blackflies in conjunction with the program on biocontrol. Salary commensurate with experience and qualifications. Send application etc. to Dr. M. Laird, Director, Research Unit on Vector Pathology, Memorial University of Newfoundland, St. John's, Newfoundland. (1673)

#### POSTDOCTORAL FELLOWSHIPS

opportunities are anticipated in the following areas of biting fly research program on biocontrol; 1. Ecology of larval blackflies; 2. Laboratory studies and culture of pathogens and parasites of biting flies, and 3. Field surveys for biting fly pathogens and parasites. Send application etc. to Dr M. Laird, Director, Research Unit on Vector Pathology, Memorial University of Newfoundland, St. John's, Newfoundland. (1707)

#### GIRTON COLLEGE **CAMBRIDGE**

Applications are invited for a SCIENTIFIC RE-SEARCH FELLOWSHIP open to women graduates and tenable for three years from October 1, 1975. The applications should be in the field of Mathe-matics, Natural Sciences, Geography and allied

Each Fellowship is to the value of £1,000 per annum and pensionable. Particulars are available from the Secretary to the Council, Girton College, Cambridge, to whom applications should be sent by January 8, 1975.

#### UNIVERSITY OF NEWCASTLE UPON TYNE **FELLOWSHIPS**

Applications are invited for the following Fellowships (tenable for two years from October 1, 1975) from persons who have shown themselves able to carry out original research. The value of each Fellowship is £2,118 in the first year and £2,247 in the second year.

SIR JAMES KNOTT FELLOWSHIPS

# Two Fellowships available in any faculty. EARL GREY MEMORIAL FELLOWSHIP

One Fellowship tenable for research in any faculty. As a general rule the Fellowship will be awarded to candidates who have graduated from the University of Newcastle upon Type or from King's College in the University of Durham.

Further particulars and application forms (which must be returned by December 31, 1974) may ge obtained from the Registrar, University of Newcastle upon Tyne, NE1 7RU. (1761)

#### THE WORSHIPFUL COMPANY OF SCIENTIFIC INSTRUMENT MAKERS TRAVELLING FELLOWSHIP

Applications are invited for a Travelling Fellow-ship operated in collaboration with the Massachu-setts Institute of Technology. The object of the Fellowship is to advance instrument technology in the United Kingdom. It is tenable for one or two years and it will provide facilities for an approved study and/or research programme at MIT worked out in collaboration with faculty advisers. It will head to a higher degree of the Institute.

The Fellowship is open to a suitably qualified

The Fellowship is open to a suitably qualified graduate with appropriate experience who must be a British National. It is a condition of acceptance that the holder must return to British for at least two years when the Fellowship has been completed.

For a graduate who wishes to make management in the instrument industry a career, the Fellowship may be partly or wholly carried out at the Sloan School of Management, part of MIT.

The Worshipful Company of Scientific Instrument Makers will select and nominate for approval by MIT the Fellow and will finance the applicant during the tenure of the Fellowship. Married men will have the opportunity of being accompanied by their wives and families.

Application forms, which are obtainable from The Clerk, The Worshipful Company of Scientific Instrument Makers, Alliance House, 12 Caxton Street, London SW1, must be returned by November 24, 1974.

#### UNIVERSITY OF BIRMINGHAM DEPARTMENT OF EXPERIMENTAL **PATHOLOGY**

A RESEARCH FELLOW is required to join a research team under M.R.C. sponsorship presently working on carcinoembryonic antigen and cell mediated tumour immunity. Applications are invited from graduates with research qualications and experience in related fields. Salary: £2,118 to £2,412 plus F.S.S.U.

Enquiries and applications should be made to Dr. P. W. Dykes, Department of Experimental Pathology, University of Birmingham, Birmingham B15 2TJ. (1821)

#### UNIVERSITY OF CALGARY

### DEPARTMENT OF **PHYSICS**

Applications are invited from suitably qualified persons (M.Sc. or Ph.D.) for a position as Research Assistant/Postdoctoral Fellow in the field of experimental/observational AERONOMY. Interest and/or experience in near infrared attemps/persons/science accordingly. emissions considered advantageous.

Write for further information and giving resume of background to: Professor A. W. Harrison, Department of Physics, Calgary, Alberta, Canada, T2N 1N4. (1774)

#### AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for appointment to t

#### RESEARCH SCHOOL OF BIOLOGICAL SCIENCES RESEARCH FELLOW IN NEUROPHYSIOLOGY

The successful applicant will work with P. fessor R. F. Mark. Head of the Department Behavioural Biology, on problems associated with development and maintenance of inter-neuron and neuromuscular synaptic connections. In addition to electrophysiological equipment, the Department has a neuro-chemistry laboratory, facility for electron microscopy and will be developing ethological section in the near future.

Closing Date: December 2, 1974

Closing Date: December 2, 1974.

Salaries: Salary on appointment will be accordance with qualifications and experient within the range: \$A9.002 to \$12.269 p.a. Curre exchange rates are approximately \$A1: 50 \$US1.30.

Other Conditions: Tenure: Research Fellow normally for three years in the first instance we the possibility of extension to a maximum of f

Reasonable travel expenses are paid and assignee with housing is provided for an appoint from outside Canberra. Superanneation is on 4 F.S.S.U. pattern with supplementary benefits.

The University reserves the right not to make an appointment invitation at any time.

Prospective applicants should write to the Assistant of Commonwealth Universities (Appls).

Gordon Square, London WCIH OPF for further particulars before applying.

(1805)

#### UNIVERSITY OF RHODESIA FACULTY OF MEDICINE

#### DRUG METABOLISM RESEARCH

The University of Rhodesia Faculty of Medici invites applications for pre- and postdoctor awards to undertake research in drug metabolis or associated areas of pharmacology. Successicandidates could be considered for events permanent appointment in a new department.

Research Fellow salary scales are approximate equivalent to US\$5.500 to US\$13,900 (£2.300 £5.900) with liberal fringe benefits, health insurant and return fares.

One permanent position available for physiologic Applicants should submit brief resume and quest further details from Head. Department Physiology, Godfrey Huggins School of Medicat University of Rhodesia. P.O. Box MP 167, Mon Pleasant, Salisbury, Rhodesia.

#### TWO POST-DOCTORAL FELLOWSHIPS IN THE EARTH AND PLANETARY SCIENCES

The Lamont-Doherty Geological Observatory of Columbia University invites scientists interested in any field of the earth and planetary sciences to apply for the following Fellowships.

Two Post-Doctoral Fellowships, each awarded for a period of one year (extendable to two years in special instances) beginning in September, 1975, with a stipend of \$12,000 per annum. Completed applications are to be returned by February 15. Application forms may be obtained by writing to the Director, Lamont-Doherty Geological Observatory, Palisades, New York, 10964

Award announcements will be made March 31, 1975

#### UNIVERSITY OF RHODESIA DEPARTMENT OF AGRICULTURE RHODESIA CATTLE PRODUCERS RESEARCH FELLOWSHIP

Applications are invited for the above-mentionel Fellowship from Honours graduates in Agriculture, Blochemistry or Mammalian Physiology who wish to work on the endocrinology of bovine fertility. Preference will be given to condidates who are interested in developing radioimmunoassays for steroid hormones. The Fellowship is tenable for

three years.

Salary Scales and Conditions: Appointment will be within one of the following grades; entry point dependent upon qualifications and experience. (Approx. Stg. Equiv.): Junior Research Fellow Grade II £2,967 by £151 to £2,807: Research Fellow Grade II £2,967 by £142 to £3,109. Passages and allowance for transport of effects on appointment. Housing allowance, Superannuation and Medical Aid Schemes.

Applications: (6 copies) giving full personal

Aid Schemes.

Applications: (6 copies) giving full personal particulars (including full names, place and date of birth, etc.), qualifications, experience, publications and giving the names and addresses of three referees should be submitted by November 30, 1974, to the Assistant Registrar (Science), University of Rhodesia, P.O. Box MP.167, Mount Pleasant, Salisbury, Rhodesia. An additional copy should be sent to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF, from whom further details may be obtained. (1796)

# UNIVERSITY OF SURREY DEPARTMENT OF METALLURGY AND MATERIALS TECHNOLOGY

#### RESEARCH IN ADVANCED MATERIALS

Applications are invited for a Research Appointment. The Department offers excellent facilities for research and there will be close interaction with industry.

FUNDAMENTAL MECHANISMS IN SHAPE-MEMORY ALLOYS

A post doctoral Fellowship or Graduate Research Assistantship on a 3 year S.R.C. grant to study the origins of shape-memory in alloys, Such materials are now being exploited commercially and present an exciting new field of research. The work will involve advanced metallography and physical property measurements on alloys exhibiting appropriate martensitic transformations. The salary scales are:
Post doctoral Fellows: £2,247 to £2,580 (plus Threshold Payments)
Graduate Research Officers: £1,212 to £2,385 (plus Threshold Payments).
Curricula vitae and the names and addresses of 2 referees should be sent to:
Professor M. B. Waldron
University of Surrey
Department of Metallurgy and Materials
Technology
Guildford
Surrey GU2 5XH

Guildford Surrey GU2 5XH

(1832)

(1847)

#### UNIVERSITY OF WARWICK RESEARCH FELLOW IN THEORETICAL PHYSICS

Applications are invited for a two-year appointment as a Postdoctoral Research Fellow in the Theoretical Physics Group. The successful candidate will work on the theory of hopping conductivity in amorphous materials with Professor P. N. Butcher. Salary in the range of £2,118 to 22.580 p.a. plus threshold payments and participation in F.S.S.U. Applications as soon as possible with a full curriculum vitae to the Academic Registrar, University of Warwick, Coventry CV4 7AL, quoting Ref. No.: 10/A/74 (1841)

### UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

#### M.Sc. RESEARCH STUDENTSHIP

Applications are invited for a research studentship to investigate the relationship between the formulation of certain dosage forms and their biological effects. The student will be expected to register for an M.Sc. degree. Payment will be at current S.R.C. postgraduate rates. Applicants, who should preferably be graduates in Pharmacy, should contact Dr A. T. Florence, Department of Pharmaceutical Chemistry, University of Strathclyde, Glasgow GI 1XW.

#### JAMES COOK UNIVERSITY OF NORTH QUEENSLAND RESEARCH FELLOW CORAL REEF BIOLOGY

The successful applicant will be expected to take an active interest in coral taxonomy, coral growth, faunal assemblages colonisation and recruitment. The project requires experience in scuba diving, transect work and a knowledge of, or keen interest in, underwater photography. Applicants must have a doctorate and appropriate research experience. Previous experience in tropical areas would be an advantage.

Salary within the range \$A9,002 to \$A12,352

would be an advantage.

Salary within the range \$A9,002 to \$A12,352
per annum plus a locality allowance of \$A142 p.a.
for a married male or \$A71 p.a. for a single
appointee. The Australian Research Grants Committee has provided funding to support the research fellowship up to December 31, 1975, in the
first instance. Conditions of appointment provide
superannuation and allowance towards travelling
and removal expenses on appointment.

Further details and application forms obtainable

Further details and application forms obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WCIH

Applications clos November 29, 1974. (1839)

### UNIVERSITY COLLEGE OF NORTH WALES BANGOR SCHOOL OF

PHYSICAL AND MOLECULAR SCIENCES Applications are invited for a

#### SCIENCE RESEARCH COUNCIL POSTDOCTORAL FELLOWSHIP

in the School of Physical and Molecular Sciences The successful candidate will be required to work under the general supervision of Dr G. Parry Jones on the dielectric aspects of high electric field effects in molecular systems. It will be an advantage to have either experience in the dsign and operation of equipment for use in the region 1-200 MHZ or an interest in biomolecular

The fellowship which may be held for up to two years has a salary of up to £2,247 by £165 per annum plus F.S.S.U. benefits (depending on age and experience).

and experience).

Applications (two copies) giving details of qualifications, research experience and the names and addresses of two referees, should be submitted by not later than November 30, 1974, to the Secretary and Registrar, University College of North Wales, Bangor, from whom further particulars may be obtained.

(1843)

#### AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for appointment to the following:

#### RESEARCH SCHOOL OF BIOLOGICAL SCIENCES POSTDOCTORAL FELLOW MOLECULAR BIOLOGY UNIT

The Unit (Head: Dr H. Naora) is exploring the regulatory mechanism of translation in animal cells, particularly the molecular mechanism which underlies the ability of ribosomes to specifically recognised mRNA or messenger-like RNA. This includes the problems of HnRNA-mRNA pre-cursor relationship and the interaction of these macromolecules with ribosomes. Applicants should have experience in the field of RNA and ribosome research. Some experience in developing eggs and also tumor cell research would be an advantage. advantage.

Closing date: December 14, 1974.

#### **FACULTY OF SCIENCE** LECTURING FELLOW

DEPARTMENT OF BIOCHEMISTRY

DEPARTMENT OF BIOCHEMISTRY

Applicants should have a Ph.D. degree or similar qualification. The appointee will be asked to contribute to teaching programmes for graduates, honours students and undergraduate students. It is desirable, but not essential, that the appointee should have had experience in the broad field of metabolism. The appointee will be required to take up duty as soon as possible. Closing date: December 9, 1974.

Salaries: Salary on appointment to the post will be in accordance with qualifications and experience within the ranges: Lecturing Fellow \$A9,002 to \$A12,352 p.a.; Postdoctoral Fellow \$A9,002 to \$A12,352 p.a.; Postdoctoral Fellow \$A9,002 to \$A12,269 p.a. Current exchange rates are approximately \$A1—56new pence; \$US1.31.

OTHER CONDITIONS: Tenure — Lecturing Fellow is normally for three years in the first instance with possibility of re-appointment for a further two years.

Reasonable travel expenses are paid and assistance with housing is provided for an appointee from outside Canberra. Superannuation is on the F.S.S.U. pattern (where applicable) with supplementary benefits.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should write to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, for further particulars before applying. (1840)

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Date .....

### LECTURES AND COURSES

EUROPEAN MOLECULAR BIOLOGY ORGANISATION OPHYS

Postgraduate Course

### **Advanced Animal Virus Genetics** and Molecular Virology

An EMBO study course in Advanced Animal Virus Genetics and Molecular Virology will be held in the Institute of Virology, University of Glasgow from March 16th to April 4th 1975 under the direction of Professor J. H. Subak-Sharpe. The intensive laboratory periods will be supplemented by 15 lectures as well as specialised discussion. In addition there will be 7 special guest lectures.

The Course will be particularly appropriate for research workers in the field with three or more years postgraduate research experience as the programme will cover more recently developed techniques used in the genetic and biochemical analysis of animal virus systems, for example, triparental cross recombinants, restriction enzyme fragment analysis, or application of enucleate cells in animal virology. The fee is £90 excluding accommodation. The number of places is restricted to 16. Limited EMBO funds are available to help those applicants who could not otherwise attend.

For further details and application forms write to:

Professor J. H. Subak-Sharpe, The Course Director, The Institute of Virology, Church Street, Glasgow G11 5JR, Scotland. Closing date for applications is January 6, 1975.

(1754)

#### UNIVERSITY OF LONDON

A lecture entitled "The Development of Nutritional Research in Uganda" will be delivered by Dr R. G. Whitehead (Cambridge) at 5.30 pm on November 19 at The London School of Hygiene and Tropical Medicine, (Large Lecture Theatre), Keppel Street (Gower Street), WC1.

ADMISSION FREE, WITHOUT TICKET
Academic Registrar

(1797)

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#### **CONFERENCES**

### THE EARLY HISTORY OF THE EARTH

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### A N.A.T.O. ADVANCED STUDY INSTITUTE

Department of Geology
The University
Leicester LE1 7RH
England

April 5-11, 1975 inclusive

The purpose of this Institute will be to review current data and ideas of early earth evolution from the time of the proto-crust to the end of the Archaean. It is intended to be an interdisciplinary meeting with emphasis on the following topics:

Implications of lunar to early Earth history, the proto-crust Evolution of greenstone belts, gneissic regions, the atmosphere, oceans and life forms

Geochronology and geochemistry

Thermal regimes and deformation patterns

Reflews of the structure and evolution of continental areas Metallogeny

We hope to see an exchange of information between a wide range of earth scientists who are invited to attend and participate in discussions. For the benefit of non-specialists each major topic will be introduced by a general review lecture.

There will be a field excursion before and after the meeting to the Scourian of the mainland (April 1—4) and the Outer Hebrides (April 12—16) of NW Scotland; participants are invited to apply for places on either of these excursions.

Further details and registration forms may be obtained from Dr. B. F. Windley at the above Leicester address. (1752)

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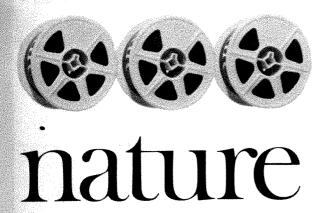
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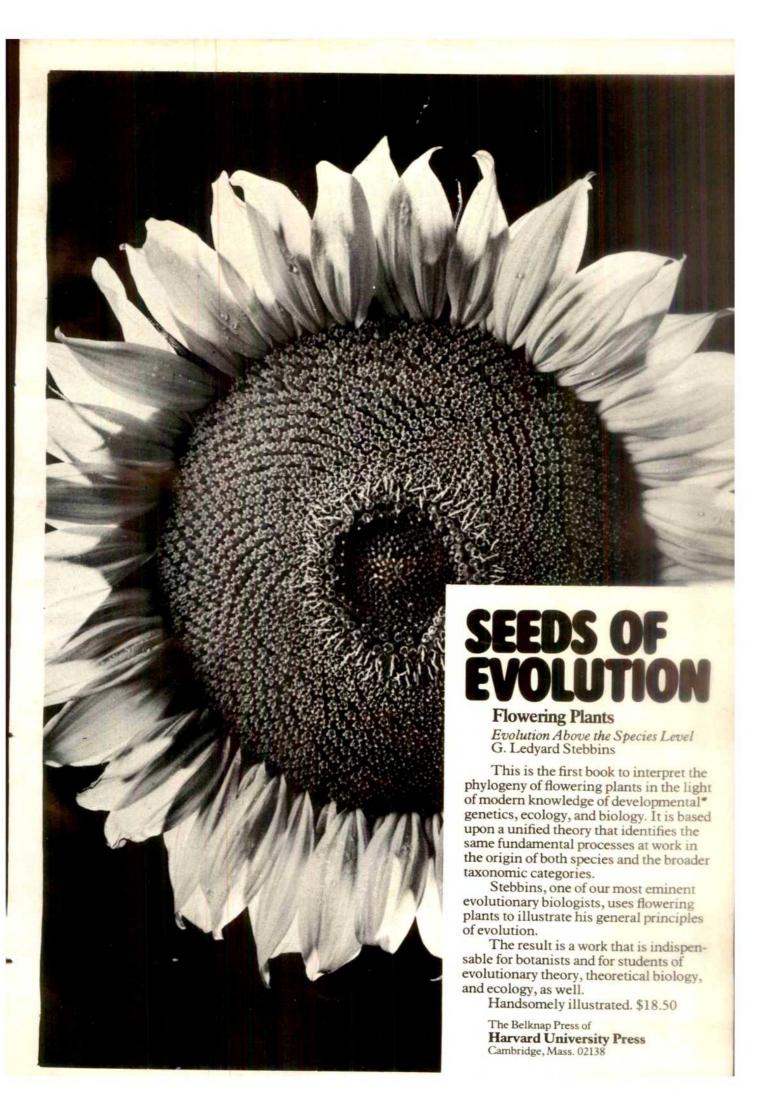
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# the first year

In its first year of publication, **Cell** has published twelve issues containing almost a thousand pages and more than a hundred articles. Already **Cell** is one of the most important journals in modern biology.

# The November and December issues of Cell include the following articles:

Shaking 3T3 Cells: Further Studies on Diffusion Boundary Effects: M. Stoker and D. Piggott

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A glacier in Magdalene Fjord, Norway. [photo by J. Allan Cash] Climate this week on pages 182, 189, 199 and 216.

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## nature

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# Getting scientists interested in policy

Science policy begins to bite—that is a clear message emerging from the annual reports of many bodies supporting science, and most recently from the Science Research Council (SRC), guardian of £70 million of British research from neurobiology to high energy physics. The message comes equally from the international organisations.

For years the last thing that most practising scientists wanted to know about was the machinery by which policy for science was evolved. After all the money generally could be relied on to roll in provided the proposal sounded plausible enough and dreary matters of policy could be left to those amiable and harmless civil servants in London, reinforced with sensible and central professors prepared to commute to endless committee meetings. We neither knew nor cared who they were, what they did in our name or how we were represented in international organisations because the funding was sufficiently pluralistic that if A regretfully couldn't provide, B probably would. And we were mercifully spared from Five Year Plans and the like, which we knew stultified Soviet science, for example. Even when a little forecasting was done and there was talk of shared facilities. we knew that if we produced the goods we would be allowed to go our own way.

The first shock came, of course, in the very late 1960s when university expansion ended and the devaluation of sterling was indeed starting to get at the pound in our pockets. But the real blows have come within the past two years when the budgets for science has been unable to keep up either with rising costs or with the ever increasing demands that scientists make on it. Added to this, universities find themselves with insufficient funds to guarantee that vacant posts can be filled. So the words priorities and planning will assume a greater significance and scientists will find themselves increasingly subject to decisions made for them by councils and committees. The words in the SRC report "... the Council have provisionally concluded ... that the priorities for astronomy, engineering and the area supported by the Science Board should be sharpened at the cost of reductions in other expensive programmes . . . " can only be a foretaste of what is to come in straitened times.

It is impossible to argue with this in principle. If the government pays, then the government has the right to assert priorities. Moreover in some respects, particularly at the moment as regards questions of manpower, one could wish for more central direction. Do we really want to see, in the absence of some corrective pressure in schools and universities, a continuation of such trends as those which lead to many astronomers and few chemists? Nevertheless the very lack of prior interest in science

policy among most scientists raises some important questions.

To what extent are those who make and implement policy responsible to the scientist whose livelihood may be threatened? The short answer is not at all, neither in terms of being elected by him nor in terms of being required to discuss decisions with him. This is hardly to say that if you call up, say, the SRC and ask for extension 1 you will not be able to speak with the chairman. You can, and he doubtless will give a civil reply. Mechanisms do not exist, however, for ensuring that the grass roots has any say whatever in the appointment of those in authority or who represent the United Kingdom internationally. No more do they exist for requiring that decisions be open to scrutiny, criticism and maybe modification in the light of this criticism.

It is not obvious that the system by which scientists are appointed rather than elected should be changed. Much dedicated work is done by people who would have no stomach for an electoral process and in the absence of party labels most electors confronted with a list of names about which they know nothing will vote at random—and in any case, who votes? But one would have greater confidence that an election was unnecessary if there were more frequent occasions on which rank-and-file scientists could listen to their representatives explaining decisions and listening to opinions. This is partly the function of the scientific press but mainly it needs some regular forum where scientists can get together.

This would be an ideal function for the British Association (BA) to perform. We have already proposed that the BA should take a more active role in making scientists more aware of their common interests by the forming of local cells, and one of the most valuable things that such cells could do would be to generate an enhanced interest in the questions of decision-making on scientific matters at governmental level. Such regular opportunities to talk about the problems at the top could hardly be other than most welcome to those who have, in the name of scientists, to try and share out a diminishing cake.

### A hundred years ago



The commotion created in th: Paris School of Melicine by the false rumour spread by the Figaro has been beyond bounds; not only was M. Wurtz, the Dean, cheered, but M. Chauffard, one of the professors belonging to the clerical party, was hooted, and unable to deliver his lecture. The disorder having been renewed in spite of all precautions taken by M. Wurtz, the School of Melicine has been closed for a month. If students again exhibit a riotous spirit, the ringleaders will be prosecuted before a Council of War; which is a lawful proceeding, Paris being placed under a state of siege.

### Weather warning: you are now experiencing a climatic change

John Gribbin has recently been visiting climatic research centres in the United States and Canada as part of a Glaxo Travelling Fellowship. In this article he discusses work now in progress, the hopes of some of the scientists active in the field, and the pressing need for more research in view of world food problems.

The name of Reid Bryson, Director of the Institute for Environmental Studies (IES) at the University of Wisconsin-Madison, is one which arouses strong response from most climatologists and meteorologists. His outspoken views on the imminence of global catastrophe and on the form of food shortages, and the degree to which he blames man's activities for the decline in global temperature over the past two decades, are either taken at face value or fairly strongly opposed—there is no middle ground.

Bryson and his group are particularly concerned with two aspects of the changing climate: determining precise quantitative parameters of past climates from analysis of pollen samples and predicting the development of present climatic trends, with special reference to the problems of food production. The former line of study causes no breast beating even among Bryson's most vociferous opponents. In its latest form, this study involves analysing pollen from the sediment layers (varves) in cores obtained from lakes in the north-eastern United States. The thickness of the varves, laid down in successive years, provide a guide to the year-by-year changes in mean climate; but that is at best a crude measure and the application of pollen analysis to the cores provides potentially the best indicator yet of how past climates have changed.

The continuous varve sample goes back for some 9,000 years—a longer and more complete record than the tree rings which have already proved so useful to climatologists. They provide more information over the whole span and there is the additional bonus of inorganic material from the sediments which provides other information about age and climate. So far, the Madison team has established a clim-

atic chronology made up of one sample per decade over the 9,000-years; work is now in progress on more detailed studies.

The facts which emerge from the analysis of past climates often encourage dramatic presentation. Bryson mentions "sharp" changes occurring within 17 years which are revealed by pollen studies. Meteorologists have argued that the atmosphere simply cannot change dramatically in such a short time, but, as Bryson puts it, "the theories must be made to fit the facts, not the other way around". And the facts he presents certainly require urgent explanation, suggesting as they do that it is possible to go from an interglacial regime (such as the present situation) to full glacial conditions in only 100 years.

According to Bryson, man's activities as an atmospheric polluter have reached a stage where that may be exactly what we are in for. Like many other climatologists, he sees volcanic activity as a prime cause of past changes, because of the effect of dust on the transmissivity of the atmosphere. Unlike some of his contemporaries, however, Bryson is convinced that man-made 'dust' is now producing a cooling trend as significant as any recorded effect of volcanic dust. In his own words "the troposphere shows clear evidence of a man-made effect since the 1940s", and this corresponds to the global cooling which has been going on since about that time. Bryson rejects the suggestion that variation in the Sun's activity could be responsible for a major part of this trend. "Don't tell me this is a major effect-how could it be physically?" was his response to my suggestion that the possibility deserved further study. This response was not entirely convincing, however, in the light of his own remarks about the need for theories to fit the facts, rather than the reverse.

Still, even if he does not go along with this particular controversial idea Bryson has plenty of publicity-worthy ideas of his own. Some of the latest work to emerge from the IES points to a relation between the incidence of condensation trails produced by jet aircraft over the North Atlantic, loss of sunlight and reduction in the euphotic zone of the oceans, a reduction in nutrients, and a delay in warming, which produces less plankton at a later time in the year than would

otherwise be the case. This delayed and decreased bloom of zooplankton is just what has been observed since 1950, selectively under the jet route, according to Bryson's figures.

But in spite of the 'gee whizzery' of such attempts to arouse public interest in the problem of climatic change there is an underlying seriousness to this work which justifies all the publicity-grabbing efforts. Bryson and his colleagues have produced projections of the fall in world food reserves which ought to be enough to shake even the dullest politician. By 1975, we could reach a situation in which only 8 to 10 days' supply of food remains in reserve—and the food distribution 'pipeline' needs 8 days' supply to keep things moving as they are ... present. This bears out calculations by the World Meteorological Organisation (see Nature, 250, 176; 1974) which highlighted the fact that there are no longer any substantial reserves of either food or unused farming land available. And it is his efforts to publicise this situation which are seen by other climatologists as Bryson's chief contribution to the problem of tackling present climatic change.

There is certainly a growing public awareness in North America of the fact that something is going wrong with the weather. The driver of the airline bus in Madison commented on the early frosts which have affected local farmers drastically, and a TV weather forecaster on a Chicago station commented in passing on the "three early cold waves which haven't done much good for the food situation". Stephen Schneider, of the National Center for Atmospheric Research (NCAR) in Boulder agrees that the food situation is worrying, but stresses the sense of urgency conveyed by Bryson's work without echoing his sense of certainty.

According to Schneider, we do not know enough about the natural processes of climatic change, or about man's influence, to be dogmatic. As Francis Bretherton, Director of NCAR put it, climatic research has been the "Cinderella of the meteorological sciences for the past two decades"; at present we are still at the stage of planning and getting a programme together. But unfortunately the deterioration in climate, if it is real, will not wait for the necessary research to be carried out. Schneider sees the possibility of a severe famine situation in

Reid Bryson of IES: outspoken views on imminent global catastrophe.

Ethiopia this winter as just one example of the immediate problems to be faced.

For the past 10 to 15 years the world climate has generally been good for food production, but bad husbandry and lack of foresight has produced a crisis situation. Supply and demand are now too close for comfort; Schneider believes that even three years ago it would have been possible with good husbandry to provide for all except the most extreme weather conditions, but now we need good weather for several years simply to get back to a marginal position of safety. The problem, he says, is man-made—but in the sense of being man-made foolishness, not technological.

In the long term the situation still looks bleak to Schneider. Although the problems to be solved are within the abilities of modern technology (in terms of increased yield per acre, improved standards of living and simply better 'housekeeping') the kind of budget and effort required would rank with the present military budget of the United States. In those terms, the problems look insurmountable simply in a political sense.

In spite of this gloomy prognosis, workers at NCAR and elsewhere continue to tackle many aspects of the changing climate situation with an increasing level of activity. If we can survive the immediate crisis which has been produced as a result of the interaction of population, food supply and climate, then we will be much better armed to tackle similar problems in future. Studies such as that by Warren Washington (NCAR) of the importance of waste heat from power stations and an investigation by Canadian meteorologists into the effect of reservoirs on precipitation are typical of the kind of problems which could, and should, have been tackled ten or more years ago and which are essential if the world is to continue to support anything like the present level of population.

The Canadians—notably the Meteorological Applications Branch of Environment Canada—have an enviable reputation for foresight because they possess equations relating wheat yield to the weather in Canada in any particular year. This, however, is more an accident of history than anything else, as the Branch's Director, Gordon Mackay, is quick to point out. The entire economy of Canada is so dep-



endent on wheat that this problem was tackled from an economic viewpoint, not through any concern about world food supplies. Nevertheless, the result is that the Canadians do have an existing working technique for determining wheat yields, and this could well be adapted to forecasting crop variations in other parts of the world.

Mackay is adamant that with the limited staff and resources at his disposal the Meteorological Applications Branch must consider primarily problems which affect Canada, although, of course, he recognises that this requires some understanding of the circulation of at least the Northern Hemisphere. Indeed, Mackay says that meteorologists in Canada would not really be aware of the changing climatic situation without the speed of modern communications; in parochial terms, they have no real problem as yet, although farmers have begun to switch to crops which require a shorter growing season, to such an extent that some kinds of seed grain are now very difficult to find.

This, perhaps, points to one immediate way in which the twin problems of food and climate can be tackled even before a full understanding of climatic change is developed. Farmers around the world must give up their traditional crops if these are not producing good yields and turn to the kinds of crops which can be grown satisfactorily in the conditions now prevailing. Echoing the sentiments expressed by Schneider, Mackay said that 50% of the world food supply is wasted by bad management or des-

troyed by pests and disease, some of these, such as rust, themselves being related to climatic factors.

The outlook is certainly gloomy, unless governments do a lot more than just talk about the problems at such gatherings as the World Food Conference. And it is no use for the holders of the purse strings to expect that a sudden change of heart on their part will belatedly lead to dramatic new revelations about how climate is changing and what influence this is likely to have on food supply. As Mackay put it, the situation today in climatic research is not unlike the situation in meteorology a hundred years ago. At that time, the Canadian Meteorological Service was founded to provide storm warnings, and functioned at first more or less on the principle of hoisting a warning to tell shipping "you are now in the middle of a storm"; today, the descendants of that service can tell us "you are now experiencing a climatic change". How big that change is, which direction it is moving in and just what effect it will have on the human population of our planet are all questions which can be answered quickly only if climatic research is far greater priority than it has received so far. The overall impression gained from talking with those active in these studies today is that the effort needed is comparable only to the effort of a full scale war, and without this effort then a major 'crunch', in the form of famine, disease, war or some combination of the three, will inevitably reduce the world population drastically within a few years.

## nternational news

WHEN New Delhi was chosen three years ago as the site for the 26th International Congress of Physiological Sciences, the Indian government gave assurances that nobody would be prevented from attending on the basis of race, citizenship, religion, political philosophy, language or sex. But when the event took place last month, visas were denied to scientists from Taiwan? Portugal, Rhodesia and South Africa. and scientists from Israel were only permitted to attend after attempts to exclude them drew strong international protests.

Those events, which are of course commonplace for conferences held in the Soviet Union, have been brought to the attention of at least one influential international scientific body, with the result that India is likely to slip a few places in the list of desirable locations for scientific jamborees.

New Delhi was selected as the site for this year's meeting by the General Assembly of the Internatioal Union of Physiological Sciences (IUPS) in 1971 but it was not until this summer that it became known that the Indian government intended to deny visas to scientists from five countries with whose policies it disagreed. Although no official

### **India** barred conference delegates

reasons have been given, it is generally assumed that the Israelis were to have been excluded because India wanted to stay on the right side of Arab oil suppliers, the Taiwanese because of Taiwan's expulsion from the United Nations, the Portuguese because of Portugal's colonial policies in Africa, and the Rhodesians and South Africans for obvious reasons.

When the news reached the United States National Committee for the IUPS, it immediately called the matter to the attention of the International Council of Scientific Unions (ICSU), a major international scientific body, and asked the Foreign Secretary of the National Academy of Sciences to open negotiations with the Indian Embassy in Washington.

At the same time, a delegation of physiologists in India met with the Prime Minister, Indira Gandhi, to try

to persuade her to allow free access to the meeting.

An ICSU General Assembly, held in Istanbul a month before the IUPS Congress, passed a resolution to the effect that repeated incidences of restrictions on free communication between scientists would result in the ICSU advice to its constituent unions not to plan scientific meetings in such countries

As a result of those pressures, the Indian government relented a little by granting Israeli scientists 21-day landing permits, but it held fast on its decision to exclude Taiwanese, Portuguese, Rhodesian and South African participants. One factor in the decision to allow Israeli participation was probably the fact that three symposia associated with the congress were being held in

It turned out, however, that no Rhodesians or Portuguese registered to attend and that the one South African who applied held a British passport. (He later elected to stay away when his South African wife was refused a visa, however.) But five Taiwanese who wanted to attend the meeting were refused visas and thus were excluded from the conference.

INDIA'S Fuel Policy Committee (FPC, set up in 1971 in the Planning Commission to project a long term energy picture for the country) has finally submitted its report to the government. The Arab Israeli war of October last year had apparently caught the FPC napping; it was all set to submit its report in December 1973 but found to its chagrin a good number of its energy board comprising representayear for the FPC to surface again with a report.

design her energy policy for the next But it has also urged that priority installation of 'captive' power stations few decades on the assumption that should be given to exploration for oil coal rather than oil would be the and uranium. energy base of India's economy.

energy needs by the end of the Fifth plants for more effective utilisation of Plan period (1978-79) is as follows: this resource in the domestic and rural 135 million tonnes (Mt) of coal; 34.4 sectors. (Incidentally, gobar gas had committee's view, reserves of coking Mt of mineral oil and petroleum pro- not found any mention in the FPC's ducts; 120 billion kW h of electricity and 123 Mt of firewood, charcoal and about to submit in December 1973). because of the expected industrial dungcake.

policy, the FPC has made suggestions for administrative reorganisation; it would like the government to set up an energy commission and also an

### Indian power plan

from Narender K. Sehgal

assumptions and calculations demol-tives from the Planning Commission ished by a series of unprecedented and ministers concerned with petrolrises in oil prices. It took almost a eum, mines, railways and irrigation.

In line with its 'back-to-coal' recommendation, the committee has sug-One of the central recommenda- gested that all new fertiliser projects into fissionable fuel. tions would like to see the country should plan to use coal as feedsteck.

The FPC has strongly recommended The committee's forecast of India's popularisation of gobar (dung) gas

To give effect to a coherent energy suggested that hydroelectric schemes on five-fold within the next 20 years.

river systems to the extent of 80 to 100 million kW capacity, be investigated and installed within the next 20 to 30 years. It would also like power stations to be set up at coal pithead sites to ease pressure on movement of coal to existing distant thermal stations.

With regard to nuclear power, the FPC expects that by 1985-90 the country's uranium resources will be able to support an installed capacity of 6 to 8 lakh MW for three decades, and that by that time fast breeder reactors too will have come on line to turn thorium (which India has in great abundance)

The FPC is not enthusiastic about by industries (private or public) because, in its view, that would not be in the "overall national interest".

On energy research and development, work on conservation of coal should be given urgent attention since, in the coal are not expected to last much earlier energy projection which it was longer than 40 years or so, whereas For power generation, the FPC has growth coal requirements will increase

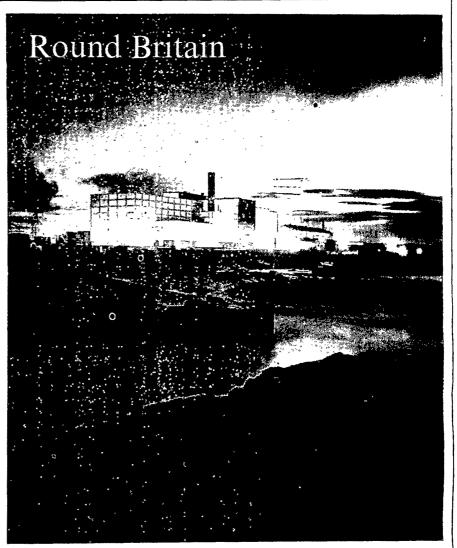
In view of the emergency cuts in public spending last December, the Science Research Council (SRC) will have in real terms 2% less money to spend in the next year, but promises in its annual report that direct grant support to the universities will be maintained at the present level—reassuring news in the present financial crisis. The real cut in expenditure rather than the planned increase of 1½% will mean, however, that the council has to defer a number of major projects. A large slice of the SRC's budget (about onethird) goes on international projects such as ESRO, CERN and the reactor at the Institut Lane-Langevin, and fluctuating exchange rates over the past year have inflated the real contribution to international programmes.

The probable cuts in university expenditure on research loom large in the SRC's thinking for the future. The cutback in university spending is likely to throw more of the burden of supporting basic research on to the SRC at a time when their funds are not increasing.

This will mean that the SRC will have to be much more selective about new projects to be supported. Provisionally, the council has decided that the growth of postgraduate studentships should not be more than 1% a year and that astronomy, engineering and the activities covered by the Science Board (genetics, enzyme technology, neurobiology and various branches of physics and chemistry which do not come under the other boards) should according to Mr R. V. Moore, Man- Congregation". Scientists predominate, be given priority at the expense of some other programmes. The establishments run by the SRC will also have to face a period of restraint to help raise resources for new facilities which only the council can provide.

Dounreay in Scotland (above) is prowill be the culmination of more than not. eight months of testing since the —for example, a tiny leakage of steam weed.

The United Kingdom Atomic Energy Authority (UKAEA) is, however, look- Sir Isaiah Berlin, the President of university has left out. Whether this ing much further ahead than the time Wolfson, the university was faced with feeling can continue depends on the when the PFR generates its designed the threat either of "wholesale migra- college's continuing ability to identify 250 MW of electricity. The first com- tion to the western hemisphere, or and support people who do not fall into



• The Prototype Fast Reactor at materials for its construction.

The big question is whether CFR1, ducing some 40 MW of heat but is not assuming it gets the green light, will be or two perks such as a High Table, the expected to feed electricity into the built in as remote a corner of the environment in the coeducational colgrid for a few weeks yet. That event country as Dounreay, Mr Moore thinks lege is idyllic by many standards and

Oxford's newest college, conceived originality?

aging Director of the UKAEA Reactor reflecting not only their preponderance Group, be started in 1977 and com- amongst graduate students but also the pleted in 1983. The present design en- many uncatered-for scientific staff visages an output of 1,300 MW but an around Oxford. Now they seem regally operating temperature of 500° C (50° C looked after; through £3.2 million of lower than that of the PFR) which Wolfson and Ford money in a building would widen the choice of possible erected on the site of J. S. Haldane's house by the river.

Even if the senior members lack one the recognition afforded to wives and families almost unique in Oxbridge. reactor went critical in March. The • "The married are viewed sympathe- Down by the river, in the specially eight months have not been without tically." With those ambiguous words built loop for parking punts, the their problems, however, even though a fellow of Wolfson College led the economic crisis seemed a thousand light these have proved to be relatively minor way into the family accommodation of years away. But can Wolfson retain its Obviously the initial into one of the secondary sodium cir- eight years ago as a means of offering nucleus of fellows and the first generacuits and blockage of the seawater a home to two of the less-well catered- tion of graduate students has a crusadinput to the steam condensers by sea- for groups-graduate students and ing zeal and is united by, if nothing university staff entitled to but not pos- else, a sense of coming together to sessing fellowships. At that time, says improve the lot of those whom the mercial fast reactor (CFR1) should, worse still, blocking legislation in the neat categories or do fashionable things.

## What effect will the new faces have?

from Colin Norman, Washington

THE results of last week's elections in the United States are simple to relate. The Democrate strengthened their hold on Congress, and they now occupy more than two-thirds of the seats in the House of Representatives and three-fifths of the seats in the Senate. (All members of the House of Representatives were up for re-election and one-third of the Senators.) The new Congress, which takes office in January, will be younger and probably more liberal than the present one, and there will be more new faces on Capitol Hill than at any time since the Second World War.

What do these changes mean for science? It is important to bear in mind two factors. The first is that because of the so-called seniority system, under which committee chairmanships are given to the longest-serving members of the majority party, the newcomers will have little direct power. Few committee chairmen will be leaving this year and therefore the people who wielded the power in this Congress will continue to pull the strings in the next. The second factor is that party labels don't necessarily mean much when it comes to voting on specific issues since there is a wide spread of political opinion among members of both parties.

Nevertheless, there are a few changes in important positions on committees which deal with science and technology, with the Joint Committee on Atomic Energy being the most directly affected. Six out of the eighteen members of that committee either lost in last week's election or will be retiring at the end of this year, and the departures include two of the most influential members -Chet Holifield and Craig Hosmer. Since virtually every member of the joint committee is now a firm supporter of nuclear power, if even as few as one or two of the vacancies are filled by environmentalists or other nuclear sceptics the result would be important in changing the way the committee conducts its affairs.

Another significant departure is that of John Davis, the Chairman of the House Subcommittee on Science, Research and Development, who lost a primary election earlier this year. That subcommittee is the focal point for deliberations on general science policy matters in the House of Representatives and it also oversees the workings of the National Science Foundation. Davis's place will probably be filled by James Symington, a young and widely respected legislator from Missouri who

can be expected to step up the pace of the subcommittee's activities. Davis was also a member of the governing board of the Office of Technology Assessment and was in line to be its chairman.

Aside from direct dealings with science and technology, the new Congress is expected to be more sympathetic to environmental concerns because several Congressmen and Senators opposed by environmental groups were defeated. Among them were eight Congressmen listed earlier this year as the House's "Dirty Dozen" by a group called Environmental Action. In addition, twelve of seventeen candidates supported by the League of Conservation Yoters were successful in the polls.

Those results have been greeted by environmental groups as proof that, in spite of the present concern about energy shortages, environmental issues are still politically potent. In particular, the results from Colorado indicate that environmental concerns there played a strong part in the election of a Senator, the Governor and at least one member of the House of Representatives. The upshot could be a setback to the government's plans to promote the extraction of oil from shale and the production of natural gas from deep deposits under the Rocky Mountains.

In the Senate election, Gary Hart, who was Mr McGovern's campaign manager in 1972, defeated Senator Peter Dominick after lambasting Dominick's voting record on environmental matters. As for the governorship, a state legislator, Dick Lamm, who had strongly supported laws to control urban growth in Colorado, defeated the incumbent governor. And in addition, Colorado voters approved a proposition on their ballot papers that before any more underground nuclear blasts can be set off in that state as part of a programme to extract natural gas from tight deposits, a referendum must first be held. The result of that move is likely to be the final blow for the Atomic Energy Commission's Plowshare Project.

Furthermore, environmentalists have hailed the election of other state governors, such as Edmund Brown in California and Edward Herschler in Wyoming, who are considered to be sympathetic to their views.

The election results may also portend some changes in the area of health, since many Congressmen supported by the American Medical Association (AMA) were defeated. One possible result may be to improve the chances that Congress will give its approval in the next two years to a meaningful national health insurance scheme. The AMA, which is vigorously opposed to

anything but a minimal health insurance scheme, poured about \$1 million into the campaign chests of Congressmen who supported its policies.

Perhaps the over-riding concern during the election campaign, however, was inflation and that will also have a direct bearing on the way in which Congress deals with some scientific matters. The Democrats have now been given a strong electoral mandate to curb inflation and they will be likely during the next two years to keep a strong hand on the federal government's purse strings, with the result that few controllable items in the budget will be allowed to grow. Energy research and development can be expected to escape the worst of the squeeze and so, perhaps, can such politically sensitive areas as cancer research. But for the rest of the science budget, a period of austerity should be anticipated.

## Soviet failure on the moon

from Vera Rich

One of the happier results of Soviet-United States cooperation in space research must surely be the increasing openness of the Soviet planners about their failures. In the early days, the possibility of failure did not exist—aborted interplanetary craft were discreetly registered under the cover-all of the Kosmos programme, and probes which failed to send back data were said to have "completed their mission" by the mere fact of effecting a landing or fly-by. The announcement of the Luna-23 failure comes with rather startling frankness.

True, 1974 has not been one of the most spectacular years for the Soviet space programme. Three out of four Mars probes failed to operate on reaching the planet, and the rather coy manoeuvering of the manned Soyuz-15 around the unmanned Salyut-3 in August caused wide speculation that a link-up had been originally intended. In these cases, however, the TASS reports did suggest certain positive achievement. However, Luna-23, launched on October 28 in good time for the Revolution Day celebrations, and hailed as part of the festal "illuminations" in a Pravda article of November 6, seems to have been officially recorded as a failure. Intended to carry out drilling operations to a depth of 2.5 m, it touched down in a rough area of the Mare Crisium, wrecked its drilling gear, and, after transmitting data for three days (which coincided neatly with the Revolution holidays) ceased functioning.

## correspondence

### Mosquito research

SIR,—The article by Narender K. Sehgal (Nature, September 20) entitled "Doubts over US in India" is misleading and misinformed. The article repeats some of the allegations which have been made during the past few months in the Indian press about current mosquito research in Delhi, but it omits many subsequent retractions in the same press.

The Research Unit for Genetic Control of Mosquitos in Delhi is a joint venture of the Indian Council of Medical Research and the World Health Organisation. The unit was established about five years ago to study the ecology of three of the major disease vectors of India, Culex pipiens fatigans, Aedes aegypti and Anopheles stephensi, and to investigate genetic mechanisms which might be utilised for their control. All three are difficult to control by conventional methods, not only in India but throughout their distribution range. The research programmes were planned in India by ICMR and WHO scientists who, collectively, represented a range of disciplines and who shared a wide experience of vector-borne diseases. It is clear from the erroneous and misleading statments on filariasis, dengue and malaria that neither Mr Sehgal nor his informants are qualified to judge the scientific aspects of the programme, which makes it all the more reprehensible that they themselves have not taken the "closer look at the related details" of the "seemingly scientific investigations" which they recommend to others. The details are readily available. A whole issue of the Journal of Communicable Diseases published in the middle of 1974 in Delhi describes the programme. There is no secrecy, as has been charged; about the work. Having been closely associated with the unit and its staff for five years, both as a consultant and Project Leader, I can give this assurance unreservedly.

The main charge in the controversy, unfortunately still current, is that the data being collected on mosquito ecology may be, or are being, misapplied in some fashion relating to biological warfare. The same charge could equally well be laid in India and elsewhere against work being done on insect vectors of plague, viral encephalitis and typhus. The results from a great deal of biological and medical research could in theory be misapplied,

but this is a poor argument for criticising studies specifically designed for the long-term benefit of the community. If the interpretation of current mosquito research presented by Mr Sehgal is genuinely believed by some non-medical Indian authorities, then there is clearly a said lack of understanding of the integrity and objectives both of local and foreign scientists in India and of international agencies.

There is nothing unusual about the financial support of the mosquito unit from US PL480 Indian funds. Scores of other research projects in India carried out by Indian scientists have been supported from the same source. The political implications that have been made have damaged relationships within the medical science community, and their continuance can only hinder progress in controlling mosquito-borne disease.

Sir Ronald Ross often expressed his frustration and discouragement when his research in India on Culex pipiens fatigans and bird malaria, which subsequently won him a Nobel prize, was misconstrued and retarded by the authorities of his day. It will be disheartening if, 75 years later, his successors suffer the same discouragement.

W. W. MACDONALD Liverpool School of Tropical Medicine, Liverpool, UK

#### **Protests**

SIR,—My letter published in your issue of August 12 set out the unequivocal policy of the World Federation of Scientific Workers on the rights of scientists to work and the procedure for implementing this policy in specific cases. Professor Janouch (Nature, September 20) does not approve of our policy and believes that "only open, public stands, protests and statements are meaningful and helpful". My own experience in working in international organisations over many years has taught me that, from the point of view of helping individuals in difficulty, not less than preserving the integrity of the organisation, an informal, low-key approach may often be more effective than the attitude he advocates.

Surely, also, before making protests and statements one should seek to discover whether the facts of the case have been fully and accurately presented. This is what our procedure tries to do and often, as in fact has hap-

pened in the particular case that led to this correspondence, new facts show the matter in a somewhat different perspective.

The case of Professor Holzer, referred to in Dr Janouch's letter, is much more serious than he claims because it is based on a law existing already in certain West German Länder and currently being prepared for submission to the Federal Parliament, whereby a whole category of people, members of certain legal parties and organisations, such as the German Communist Party (DKP), can be debarred from employment in public service posts, including posts as school teachers, judges and university teachers.

Dr Janouch also mentions the case of Academician Ivan Malek, who gave dedicated and distinguished service to the Federation over many years. Those who have heard or read addresses I have made to meetings of the Federation in recent years will be in no doubt about the high esteem and affection in which he is held in the Federation. Academician Malek was an active supporter of Alexander Dubcek and lost his posts as Director of the Institute of Microbiology in Prague and as a Vice-President of the Czechoslovak Academy of Sciences in the aftermath of the events of August 1968. Together with other officers of the Federation I made representations on his behalf at a high level with a view to ensuring that necessary facilities to enable him to continue his scientific work were made available to him. Although, however, he continued to work in the Institute he was not restored to his former position. According to information supplied to me from official quarters his work at the Institute terminated last autumn when he was three years past the usual retiring age of 60 for scientific workers who do not hold university chairs.

Academician Malek is a most distinguished and talented microbiologist who, in my opinion, still has very much to give both to Czechoslovakia and to world science. Speaking personally, therefore, I would deplore a regulation obliging a scientist to retire while still capable of first class scientific work no matter in which country it occurred. Nevertheless, one must surely concede that questions such as retirement age in a particular country are matters for the people of that country to determine.

World Federation of Scientific Workers, London, UK

E. H. S. BURHOP

## news and views

# Towards chemical topology

THE uninitiated, faced with the awesome variety of organic chemistry, its peculiar, cryptonymous nomenclature and its tendency to produce publications in twenty or thirty parts, might be excused for finding there more to excite the wonder of the natural historian than the rigours of mathematics. Neither is the practitioner sketching formulae in the odorous intimacy of the laboratory likely to ascribe much abstract significance to the cyphers which he learns to manipulate as by second nature. Yet the business of classifying and labelling the complicated ring and branched structures which figure so prominently in the modern subject has been recognised in recent years to involve far more than simple book-keeping; in fact some of the problems which arise, when stripped to their abstract essentials, connect with as yet unresolved difficulties in the area of finite mathematics usually termed graph theory.

Consider, for example, the following: can a coding system be devised which not only leads to the systematic reconstruction of an arbitrary carbon skeleton but which is essentially independent of the particular way adopted for labelling individual atoms or other distinguishing features? Alternatively, if a certain labelling system is adopted (usually, of course, that of attaching sequential numbers to the carbon atoms present) can coded representations be found from which the topological identity of two differently numbered versions of the same N-carbon structure can immediately be inferred? (Other, that is, than by investigation of all possible N! permutations of labelling.)

This problem, which shares its graph-theoretical content with several others in statistical mechanics and the theory of disordered structures, has been taken up in a number of recent papers, most notably by M. Randić of the University of Zagreb writing in the Journal of Chemical Physics (60, 3920; 1974). Professor Randić advances no rigorous proofs but suggests an ingenious algorithm by which a binary number code is assigned to each possible labelling of a given structure, the lowest number obtainable under systematic rearrangements providing both a canonical numbering scheme and an immediate comparison test for the identity of different networks of bonds. The method given seems to fall short of guaranteeing the discovery of the minimal labelling but seems to work well when tried on the common systems of saturated and benzenoid hydrocarbons. Needless to say the canonical numbering systems come out differently from those traditionally adopted. (It is notable, and amusing, that the basic tool of the Randić method—the adjacency matrix for comparable structures—is now a familiar part of O-level mathematics (see Schools Mathematics Project Book Y, 55; Cambridge University Press, 1973) though likely to seem formidable to many research chemists educated in an earlier era.)

The recent involvement of stereochemistry with topics such as graph theory, the less usual point-group geometries, the interconversion of symmetry types and the combinatorics of multiple structures, is an encouraging sign that this subject, cutting across all the main disciplines of chemistry, might be prominent in any defence of the latter

against charges of moribundity and compartmentalism.

A very timely cross section of contemporary (organic) stereochemical research, as well as a useful reminder of the heroic era of the subject is to be found in the recent van't Hoff-Le Bel centennial issues of Tetrahedron (30, Nos 12, 13; 1974). Here some fifty papers are collected covering topics so various, and occasionally bizarre, as to make selective quotation at best misleading. Among the less orthodox papers are those on "Polytopal Form and Isomerism" (E. L. Mutterties, Cornell), "High Symmetry Chiral Molecules" (M. Farina and C. Morandi, Milan/La Jolla), "Polyhedral Rearrangements Involving 5 and 6coordinate Carbon" (M. F. Hawthorne, K. P. Callahan and R. J. Wiersma, UCLA), "Knot-structures in Chemistry" (J. Boekmann and G. Schill, Freiburg), "Parity and Stereochemistry" (J. Mathieu and A. Rassat, Grenoble) and "Chemical Systematics in the Computer Design of Syntheses" (J. Blair, J. Gasteiger, C. Gillespie, P. D. Gillespie and I. Ugi, Munich). The last mentioned also involves the graph theoretics of labelling, the time in the enumeration of synthetic pathways by computer. Many exotic systems make an appearance—cyclophanes, propellanes, carboranes—among others less strange though scarcely worked out stereochemically, such as tetra-aryl methanes. A disappointment perhaps is the non-appearance of the marvellous and unique dodecahedrane, which several groups have been rumoured to be close to synthesising, perhaps the most beautiful hydrocarbon that will ever be made.

Yet the outsider looking in at such intricate and painstaking work cannot escape certain mixed feelings. Granted that there is a place for jeux d'esprit in such a collection, how important is it to the future of chemistry that someone should succeed in synthesising a molecule with a knot or a peculiar kind of hole in it or a particularly awkward linkage of rings? And, if dodecahedrane can be made, should we take comfort in the fact that rhombic dodecahedrane is manifestly impossible? In short, are these weird and wonderful aspects of stereochemistry outgrowths of a flourishing field, or hot-house creations of almost zero relevance to either the theory or the application of the subject? Should one not, after all, admit that chemistry has become little more than a complicated and private 'bead-game'? The riposte to this no doubt lies in pointing to the considerable achievements of mainstream stereochemistry—conformal analysis, the Woodward— Hoffmann rules, and the various aspects of 'kineticstereochemistry'—which have come into their own recently, while stressing the underlying biochemical relevance of much (though by no stretch of the imagination all) that has been done. This line is taken in a recent interview given by Alexander Todd (Chemistry in Britain, 10, No. 6, 207; 1974) who argues with impressive conviction, if not altogether convincingly, that with sufficient imagination and resources, genuinely new and relevant phenomena will continue to be found. Given that it is not an entirely happy thing that chemistry should be in a position of needing a 'something will always turn up' philosophy, only time and a great deal of work will prove-or disprove-such a contention.

Meanwhile, one might argue tentatively that the move towards interest in global structure, neighbour relationships and the problems of chemical identity touched on above are a proper, if delayed, response to the realisation that the truly basic problems of stereochemistry at the geometrical level were solved a very long time ago indeed by van't Hoff, Le Bel, Kekulé et al. and that a great deal of what has followed is elaboration rather than fundamental advance. Perhaps we shall know that something extraordinary has arrived when the authors of a radically new concept receive the kind of abuse which was heaped upon van't Hoff and Le Bel by chemists of the day, for daring to suggest that carbon atoms might combine in geometrically describable structures. But, if one's instincts are correct, this honour may well be reserved for someone in a field altogether remote from chemistry as we know it, and perhaps from physics too.

from our Molecular Physics Correspondent

### New light on old reflexes

THE voluntary muscles that move our limbs are full of sense organs, a fact in itself not surprising, although the use the central nervous system makes of the information they send it is perhaps more of a mystery than is the case for any other major group of sense organs in the body.

The most obvious manifestation of their action, the familiar knee jerk, obtained by striking the tendon below. the knee cap with a hammer, was described independently and almost simultaneously by Erb of Heidelberg and Westphal of Halle just on a century ago. In this reaction the tap to the tendon briefly stretches the muscle spindles (the most important of the sense organs in the muscle) and their discharge reflexly causes the muscle to twitch. Slower stretch of a human muscle that is already contracting, by forcible bending of the joint it acts on, also causes a reflex increase in contraction. Stretch reflexes of this general type were first described by Liddell and Sherrington in 1924 in animals rendered insentient by removing the cerebral hemispheres, whose muscles are unnaturally susceptible to stretch. The stretch reflex has long been a favourite object of research from the belief that it is an important and accessible element in the complex nervous mechanisms by which the muscle sense organs make the muscles, by some kind of feedback action, do what we ask of them. This belief is reinforced by the observation that stretch reflexes, as well as tendon jerks, are abnormal in neurological diseases such as stroke, in which the muscles conspicuously do not do what we ask of them.

The muscle spindles are the most elaborate sensory structures in the body other than the eyes and ears. Each consists of a bundle of fine modified muscle fibres with sense endings, sensitive to extension of these muscle fibres, wrapped around a central region. There are two different kinds of sense endings on the spindles, the primaries, connected to the central nervous system by fast conducting nerve fibres, and the secondaries, whose nerve fibres conduct at only a half or a third of the speed.

The latency of the knee jerk—the time from contact of the hammer with the tendon to the first sign of reflex activity in the muscle extending the knee—is about a sixtieth of a second, so short that the sense endings responsible must be the primaries with their fast nerve fibres. Even so, there is only just time for nerve impulses to get to the spinal cord and back. Until quite recently it was always supposed, following Sherrington's unhesitating identification of the knee jerk as a "fractional manifestation" of the stretch reflex, that the stretch reflex proper used the same sense endings and the same rapid spinal reflex pathway as the tendon jerk. This attractive but dangerous simplification is now under attack on two fronts.

First, P. B. C. Matthews discovered that the stretch reflex in the soleus (a muscle extending the ankle) of the cat was larger than the reflex contraction obtained by vibrating the tendon, a mode of stimulation that is believed on good grounds to excite the spindle primary endings powerfully and selectively. This strongly, if indirectly, suggests that the secondaries take part in the stretch reflex. There is no reason why they should not, for the stretch reflex has long latency components as compared with the tendon jerk. Now Kirkwood and Sears in this issue of *Nature* (page 242) present the crucial evidence that the nerve fibres from spindle secondaries, on entering the spinal cord, excite directly (monosynaptically) the motor nerve cells of the muscle in question (in these experiments a muscle of the rib cage). They have similar evidence for the secondary endings in cat soleus muscle (*J. Physiol, Lond.*, in the press). The involvement of the slow-conducting spindle secondaries in the stretch reflex of the decerebrate cat can now scarcely be doubted.

On the second front, Marsden, Merton and Morton (Nature, 238, 140; 1972; Lancet, i, 759; 1973) working on human subjects, found that in some muscles, for example the muscle that bends the top joint of the thumb, only long latency components are present in the stretch reflex. Following suggestions by Hammond and by Phillips, they argued that in such muscles the stretch reflex may not be spinal at all, but may travel over a pathway to the cerebral cortex and back. This theory may seem far-fetched at first sight, but trans-cortical reflexes from the limbs are not, in fact, a new idea in either physiology or neurology. A number of observations already fall into place on the transcortical theory of the stretch reflex, but much will have to be done to establish the theory firmly. In particular, there remains at present the possibility that the latency is long because only the spindle secondaries (to the exclusion of the primaries) take part in a spinal reflex. Results from muscles of the shoulder should throw light on this question.

These two new notions, that the secondary spindle endings take part in stretch reflexes and that some stretch reflexes may have a cortical reflex arc (notions, incidentally, by no means mutually incompatible), have a twofold significance. First, in clinical medicine, neurologists have long been puzzled that the stretch reflexes (measured as the resistance or 'tone' felt in a limb during passive movement) and the tendon jerks, which physiologists originally told them had the same mechanism, did not always go hand in hand when they altered in disease, and sometimes indeed changed in opposite directions. Now that there are at least two possible ways in which the mechanism of tendon jerks and stretch reflexes may differ, the way is open for a resolution of these problems and for a completely new approach to the whole nature of 'tone' in normal muscle and its alterations in spastic and other abnormal states.

More generally, any new information about the detailed mechanism of the stretch reflex is particularly welcome at this time when the work of Marsden et al. already referred to on the servo-like stretch-reflex-based responses of human muscles during voluntary movements has turned up several other new phenomena not foreshadowed in animal work on the stretch reflex and badly in need of explanation.

from a Correspondent

# An uphill route for climatic cycles?

Ir climate is the summation of weather over a period of time, then it makes sense to predict climatic changes in the same way as weather—that is by looking at present sequences of events and assuming that similar sequences will persist in the future. Such a method has been applied principally by Lamb and it is one of the concerns of his now rescued Climatic Research Unit (Nature, 251, 568 1974). But the detection of cycles alone does not indicate the causes of either regional or global climatic changes As realistic experiments are clearly impossible and

computer models cannot yet account for present climatic features, never mind their changes, the only practical approach is to define as closely as possible the nature and wavelength of climatic cycles and to compare them with any cyclical phenomenon that may alter climate. Lamb, for example, demonstrated that there was no strong correlation between climatic change and volcanic activity (Phil. Trans. R. Soc., A266, 425; 1970). If correlations can be established, causal relationships can be investigated bearing in mind that the two factors could be indirectly linked through some other variable.

But climatic records are of only limited range and difficult to quantify—Tacitus's description of the British climate in 75 BC as "objectionable, with frequent rains and mists, but no extremes of temperature" bears distinct resemblances to the British summer of 1974 AD, but cannot be taken to mean that we are at the same point of a climatic cycle! The only quantitative climatic measure for periods prior to meteorological measurements is that of temperature determined from the ratio of 18O to 8O now trapped in ice on Greenland and Antarctica and in skeletal remains preserved in sediments. This ratio can be measured with a high precision, but both situations raise difficulties about how the ratio should be interpreted as temperature. The snowfall over the Antarctic comprises a large amount of redeposited snow and the oxygen ratio in marine skeletal organisms is strongly affected by changes in salinity as well as by temperature. The oxygen ratios in ice sheets can be dated by short-lived isotopes or simply counting summer/winter layers—usually starting from the commencement of nuclear weapon testing which now provides an easily detectable marker horizon. But increasing compaction with recrystallisation tends to destroy the accuracy of counting and this method can only go back reliably for a few thousand years. Fossil foraminifera and other organisms can however be used to extend temperature determinations well into geological time and can be dated by standard geological methods, thereby allowing the detection of long-term cycles.

What about the factors that may alter climate? As Chappell says on page 199, there has been general acceptance that cyclical changes in the degree of solar radiation received at the Earth's surface occur because of long-term changes in the Earth's orbit. The periodicity of these changes was largely established by Milankovitch in 1938 and, assuming no changes in the solar radiation 'constant', it is possible to determine precisely the changes in the amount of insolation received at the Earth's surface. Calder, on page 216, re-evaluates Milankovitch's hypothesis in the light of revised insolation tables and concludes simply that these orbital perturbations are the first-order control on glaciation. But as Chappell points out, many critics have claimed that the radiation changes associated with these astronomical factors are very small and the direct effect of insolation may be insignificant compared with indirect factors. Chappell has previously shown many earlier correlations to be spurious, but now provides new and revised evidence for a degree of correlation between orbital perturbations, sea-level changes and palaeotemperatures for the last 250,000 years. His conclusion is also that

### More putative human tumour viruses

In this issue of Nature (page 247), McGrath, Grant, Soule, Glancy and Rich describe the release from a human breast carcinoma cell line (MCF-7) of virus particles that they believe may be human mammary tumour viruses. The particles have all the standard biophysical and biochemical properties of RNA tumour viruses, and virus-producing cells react specifically in indirect immunofluorescence tests with antiserum against murine mammary tumour virus. These findings support previous reports by Schlom, Axel, Spiegelman and their colleagues and by Moore's laboratory that virus particles related to murine mammary tumour viruses can be detected in human milk and in breast carcinomas. Unfortunately virus production from the MCF-7 cell line is rather poor and fickle, and this has hampered a more thorough analysis of the particles.

The sceptical reader will wonder whether the MCF-7 cells are really human cells and if so whether they are really derived from a breast carcinoma rather than a common laboratory cell, such as HeLa. On these points, McGrath et al. stand on reasonably firm ground, for in a previous publication (J. natn. Cancer Inst., 51, 1409; 1973) Soule, Fazquez, Long, Albert and Brennan characterised the MCF-7 cell line in some detail. MCF-7 cells are derived from cultures of a pleural effusion of a patient with metastatic mammary carcinoma. The subtetraploid cells seem to have a human chromosome pattern, although banding patterns have not been examined; they have a ribosomal RNA profile resembling that of human cells, and they carry human cell surface antigens and human glucose-6-phosphatase isozyme. MCF-7 cells show three features characteristic of mammary gland cells: cytoplasmic receptors for 17βoestradiol, synthesis of lactalbumin, and the formation in monolayer culture of epithelial 'domes' similar in morphology to those previously described by McGrath in murine mammary tumour cultures. Not all subclones of the MCF-7 cell line release virus particles. There

remains the possibility, of course, that although the cells seem to be genuine human mammary cancer cells, the virus may be a laboratory contaminant. But McGrath et al. feel that they can distinguish the MCF-7 particles from murine mammary tumour viruses, and there is no relationship at all with murine leukaemia virus.

In a paper published in the November 1 issue of *Nature* (252, 78), Lewis, Tannenberg, Smith and Schwartz suggest that an antigen detectable on lymphocyte membranes of systemic lupus erythematosis (SLE) patients is related to a C-type viral antigen. Antiserum prepared against the C-type virus produced by murine plasmacytoma SP 104 reacted in immunofluorescence tests to SLE lymphocyte membranes, but not to lymphocytes of normal subjects. Moreover, the serum of an SLE patient also reacted to SLE lymphocytes and this reactivity was specifically removed by absorption with gradient-purified SP 104 virus. Thus there seems to be a common antigen between SP 104 virus and human SLE cells.

SP '104 cells have a curious history and interesting properties. The plasmacytoma arose following inoculation of cell-free filtrates of canine SLE cells into CAF1 mice (Lewis et al., J. clin. Invest., 52, 1893; 1973). Yet the SP 104 virus stock includes a leukaemia virus containing murine gs-1 antigen. The SP 104 cells produce antibody against double-stranded DNA, as is found in SLE patients, and inoculation of SP 104 virus into normal mice or dogs induces the appearance of anti-nuclear antibodies. Thus the canine cell-free extracts and the SP 104 virus induce symptoms resembling SLE in recipient hosts. It is not known whether SP 104 virus contains canine as well as murine components; neither is it known whether the relation of SLE cell membrane antigens to viral antigens is specific to SP 104 virus alone. Nevertheless these studies do implicate a virus in the aeteiology of SLE, and they suggest that the human, canine and experimental murine forms are closely R. A. Weiss related.

astronomical factors are of prime importance, but the way in which world-wide climatic changes occur are very much more complex than indicated by Calder.

Such considerations are not merely of academic interest-they become vital as large-scale technology begins to alter the environment. What is the likely olimatic effect of diverting Soviet Arctic rivers southwards for irrigation projects? What would be the effect of a permanent ice-free channel for the movement of oil from Arctic Canada? What is the climatic effect of pollutants in the stratosphere? These questions will not be answered until a better dating system for the Quaternary enables the various cycles to be defined. Then the statistical validity of the many possible correlations can be tested and their causative relationship evaluated.

D. H. TARLING

## Plants with talent for breeding or competing

from Peter D. Moore

POPULATION ecologists have made considerable use of the concept of r and Kselection, which was introduced by MacArthur and Wilson (The Theory of Island Biogeography, Princeton University Press; 1967). When intraspecific competition (and, therefore, densitydependent mortality) is low, natural selection operates chiefly through its density-independent component, selection. Taxa associated with such situations are said to be r selected. Conversely, when intraspecific competition and density-dependent mortality are high, K selection is operative. Theoretically, r selection should favour organisms which can breed rapidly, and K selection those which can most efficiently exploit a resource. Pianka (Am. Nat., 104, 592; 1970 and 106, 581; 1972) has pointed out that no organism is completely r selected or K selected, but lies at some point along a continuum between these extremes.

Investigations into selective pressures in populations of organisms have mainly concentrated upon animals, but Gadgil and Solbrig (Am. Nat., 106, 14; 1972) suggested that selective pressures took a similar form in populations of dandelions (Taraxacum officinale) in different types of grassland at Ann Arbor, Michigan. Since this species is extremely plastic in its morphology it was necessary to use isozyme patterns in leaf extracts to determine genetic biotypes. Four such variants were found, one (A) being associated with the most highly disturbed situations, another (D) being found in stable, undisturbed situations and the other two being intermediate. The series seemed to show a trend from r to k selection.

Solbrig and Simpson (J. Ecol., 62, 473; 1974) have now published some experimental data which support this suggestion. Taxa which have been subjected to r selection would be expected to expend a larger proportion of their energy resources in reproductive effort in order to exploit rapidly an unsaturated (low competition) environment. Energy expended per seed, however, need not be high because of the lack of intraspecific competition. Biotype A was found to produce an avovage of 25.3 seeds per plant with a mean seed weight of 0.32 mg; biotype D bore 8.2 seeds per plant with a mean seed weight of 0.44 mg. These extreme biotypes thus fulfil the theoretical requirements of r and K strategists in this respect. One would further expect a Kselected taxon to be a more effective competitor under high density conditions than an r-selected one. This was tested by growing the two biotypes both alone and in combination. When in pure culture there was no significant difference between the biotypes in terms of dry weight increase over 90 weeks. When grown together, however, type D always produced the heavier plants and had the lower mortality rate; it can therefore be regarded as the stronger competitor of the two.

These two extreme biotypes thus demonstrate adaptation apparently in response to differing pressures of r and K selection in their two sites. It is not possible, however, on the basis of these experiments, to determine whether these forms of selection are the most influential ones at work upon the dandelion genomes. Habitats in which r selection is operative are frequently subjected to severe edaphic and microclimatic stresses which could have an overriding selective influence on survival. K-selection situations on the other hand experience high levels of interspecific as well as intraspecific competition, which could call for rather different adaptive mechanisms. The apomictic nature and therefore the genetic homogeneity of dandelion biotypes will make them ideal material for the investigation of these further problems.

# Meteor rates, volcanoes and the solar cycle

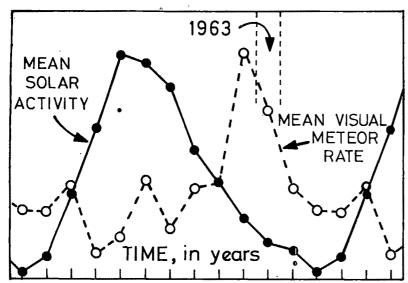
from David W. Hughes

A WORLD-WIDE increase in radar meteor echo rates occurred in 1963 and the cause of this has been puzzling scientists ever since. These echoes are obtained by reflecting short (100 µs) pulses of radiation (in the tens of MHz region) from the ionised trains produced in the

upper-atmosphere (at heights of 80 to 100 km) by incoming meteoroid particles. The number of echoes seen in 1963 was a factor of 1.5 to 2 greater than in previous and subsequent years and this increase was recorded in Christohurch, New Zealand<sup>1</sup>, Ottawa, Canada<sup>2</sup> and Onsala, Sweden<sup>3</sup>, so instrumental effects can be ruled out. The effect was periodic, in New Zealand occurring during the winter (June, July) and recurring on successive years with reduced magnitude. In the Northern Hemisphere the effect occurred initially in May 1963 but on following years recurred in the winter months, January-March. The Swedish data showed a rate increase for all echo duration. Canadian data however indicated that the anomalous increase was restricted to the smaller meteors. The rates were also proportionally higher throughout the day'.

Kennewell and Ellyett of Newcastle University, New South Wales have reconsidered the possible causes of the rate, increase in a recent article in Science (186, 355; 1974). They divide the possible causative agents into extraterrestrial and terrestrial.

Consider the extraterrestrial possibility first. There are no reasons why the influx of meteoroid dust to the Earth should not vary from year to year. For example certain meteor showers are distinctly periodic in their activity, the Leonids and Giacobinids being perfect examples. Showers are fed by the decay of the parent comet and it takes a considerable time for the new meteoroids to move around the comet orbit and form a complete, uniformly dense toroid of dust in space. The loop formation times of the Ouadrantid and Perseid meteor streams are for example 330 and 4,000 years respectively. The activity of streams which are young and not completely formed is expected to vary with the periodicity of the original comet (33 and 6 years respectively for the Leonids and Giacobinids) the dust still being closely grouped around the comet. A considerable proportion of the sporadic meteoroid background (~30%) is made up of minor streams and therefore the mean periodicity of these streams might be reflected in the total influx rate. But the 1963 increase extended over a period of some months which is incompatible with the period of activity of most meteor showers, usually less than a month. Also the fact that the recurring annual variations after 1963 were 6 months out of phase in opposite hemispheres cannot be explained. And the form of the monthly mean diurnal variation remained unchanged—this being something which would vary decidedly if a shower was active. Kennewell and Ellyett conclude that the rate in crease in 1963 was not



The mean visual meteor rate as a function of time throughout a solar cycle. The rate increases by a factor of two for the two years near solar minimum, being approximately constant over the rest of the cycle.

properties of the upper atmosphere change in 1963, resulting in the improved detection of meteors? The two

caused by an increased particle influx.

Is the cause terrestrial? Did the

main atmospheric parameters which affect meteoroid ablation are density and scale height. Now a uniform increase in atmospheric density down to meteor heights would cause no increase in the number of meteor echoes since the entire meteor population would simply rise in height. This would change the average range of the echoes. McIntosh<sup>4</sup> found a change in range of about 3 km in 160 km in 1963, a change which would have an insignificant effect on the rate. The initial radius of a meteor train varies as a function of height, also the reflected power from a train decreases exponentially with its radius. This produces a height ceiling effect, the echo height distribution being curtailed at the top. Smaller meteoroids which burn out at these great heights will be observed in greatly reduced numbers. But a simple increase in

observed rate remains the same. If the density gradient varies, as a result, for example, of a change in scale height the effect is more complex. First a larger train electron line density will result from a particular meteoroid thus allowing smaller meteoroids to come within the radar detection range. Second, a decrease in scale height will decrease the length of the train, making it more difficult to detect and will tend to cancel out the previous gain.

density just raises the height ceiling

with • the mean height, while the

Kennewell and Ellyett propose that the meteor rate increased because the scale height around 90 km changed after the massive injection of volcanic dust into the upper atmosphere by the eruption of Mount Agung on the island of Bali (8°S, 155°E) in March 1963. This dust was mainly concentrated at a height of 20 km but also formed a minor layer in the mesosphere where, by absorbing solar radiation, it led to the heating of the immediate atmosphere, a lowering of density in the region and a subsequent increase in the atmospheric density gradient higher up. The lag between the southern and northern hemisphere rate increases is thought simply to be due to dust transport times. Kennewell and Ellyett also show that total atmospheric dust content measurements made in Australia correlate with the meteor rate and that the biennial oscillation of the equatorial stratospheric winds could inject small particles into the mesosphere on alternate years and produce a twoyearly variation in meteor rates.

A cautionary note must however be sounded because 1963 is not the only year of excessive meteor rates. As Ethiopian rainfall, thunderstorm activity and the quality of claret vary with the solar cycle why shouldn't the meteor rate? This question was answered in the affirmative by the Czechoslovak astronomer Bumba in 1948 (Bull. Astr. Inst. Czech., 1, 93; 1949). He calculated, among other things, the mean annual rate of visual meteors during the period 1844-1933 as a function of position in the solar cycle and his results are shown in the figure. He also found that the ratio of bright to faint meteors and the mean observed geocentric velocity5 varies with the solar 11-year periodicity. There is an inverse correlation between solar activity and visual meteor rate, the rate maximising near solar minimum. This was also mentioned by Lindblad3 who found that the height of first appearances of meteors from a given shower remained around 110 km whereas the average end point rose from 85.2 km in 1956 to 96.0 km in 1963 -a change of 11 km. Lindblad also

found the 1953 rate considerably higher than the average.

If the increase around 1963 is just a solar cycle effect (a deduction supported by the work of Bumba and Lindblad) and not a one-off affair caused by a volcanic explosion (and it can be seen from the figure that 1963 coincides exactly with visual rate increases seen between 1844 and 1933) then similar increases should be found at other times of solar minimum, for example during 1972-73. So now is an excellent time for visual observers to peruse their records to see if this increase occurred. Also the experts in meteor ablation theory and upper atmosphere physics should try and work out why the solar cycleinduced variation in atmospheric scale height has such an effect on observed meteor rates. They could also investigate whether some unknown factor affecting meteor physics and ion chemistry varies with an 11-year periodicity. As the meteor rates are proportionally higher during the day perhaps the decrease in solar X-ray and ultraviole flux at solar minimum have an effect.

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### **Pinned** superfluid vortex

from P. V. E. McClintock

A QUANTISED vortex line stretched between definite pinning sites in superfluid helium has apparently been observed for the first time by Jost, Bogatin and Weaver of the Massachusetts Institute of Technology (Physics Letters. 49A, 147; 1974).

Liquid helium four below its so-called lambda transition temperature at 2 K behaves in many ways as though it were a mixture of two distinct fluids: a normal component which has similar properties to ordinary liquids; and a superfluid component which, having zero viscosity, is able to flow effortlessly through channels of vanishingly small dimensions and is thus responsible for the well known and very peculiar properties exhibited by the liquid.

One of the oddest features of the superfluid component it is that it cannot rotate in a conventional manner. There is excellent theoretical and experimental evidence showing that, if a container of superfluid helium is slowly rotated, the liquid remains at rest with respect, presumably, to the fixed stars, no matter how long the experiment is continued. If the angular velocity of the container is further increased, however, then it may become energetically favourable for one or more vortex lines to appear in the liquid. Each of these consists of a narrow core, oriented parallel to the container's axis of rotation, and around which the helium flows with a tangential velocity which is inversely proportional to the distance r from the core. The strength, known as the circulation, of each vortex is quantised in units of h/mwhere h is Planck's constant and m the mass of a helium atom but, so far, only singly quantised vortices have ever been detected.

Arguments based on minimising the total energy suggest that a single vortex line in a smooth container will lie along the axis of rotation, that two vortices will circle around each other under influence of their mutual flow fields, and that larger numbers of vortices will form a hexagonal lattice rotating with the container. But the recent elegant photographic experiments of Packard and Sanders (Phys. Rev. Lett., 33, 280; 1974) have shown that this does not seem to be the case in practice: the vortex cores seemed to be arranged in no definite pattern and indeed to wander aimlessly relative to the container while still presumably remaining parallel to its rotational axis.

The MIT group employed a container with a pair of definite pinning centres, designed to detect the appearance of a single vortex. The helium was contained in a 2.5 mm diameter cylinder at each end of which, positioned on the axis, was a needle, the two points being separated by about 5 mm. There were two reasons why it was expected that the first vortex would form between the needle points: first, even in a flat-ended container, a single vortex minimises its energy by being on the axis of rotation; and, second, by spanning the space between the needles, the vortex could be shorter, and thus of lower energy, than in any other feasible position. To detect the formation of a vortex line, one of the needles

was used as a field emitter, injecting electrons into the liquid, while the other acted as a collecting electrole. Because vortex lines are able to trap and hold electrons, it was hoped that there would be a sudden change in the collector current when the vortex appeared.

As the rotational speed of the chamber was gradually increased from zero, a discontinuous decrease in the collector current was indeed observed, and it occurred at approximately the expected angular velocity. Although the mechanism responsible for the fall in collector current is still not understood in detail, the authors ascribe the phenomenon to the formation of a singly quantised vortex line strung between the two needles. If they are right, then it will be the first time that a pinned vortex, long a familiar concept in connection with superconductors, has been observed in superfluid helium.

A particularly interesting suggestion by the MIT group is the possibility of using the same apparatus to search for a vortex line having two or more quanta of circulation. They argue that, if the separation of the needles is considerably less than the length of the chamber then, as the rotational speed is increased, it must be energetically preferable to add more quanta of circulation to the existing pinned vortex rather than creating further singly quantised lines which would have to span the whole length of the cylinder. Their preliminary attempts to detect such a double-strength vortex have been unsuccessful. This is perhaps because of the randomly orientated tangles of vortex lines which are believed to be generated by electrons in the large electric field close to a field emitter and which would be likely to interfere with the stability of the main. rotationally created, vortex. If this effect can be suppressed, however, perhaps by conducting the experiment under pressure when it is known that electrons have much less tendency to create vortex lines, some interesting new phenomena may be anticipated.

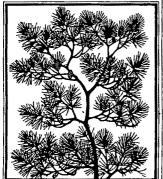
## On the trail of epidemic hepatitis

from Arie J. Zuckerman

INFECTIOUS or epidemic hepatitis, now referred to as hepatitis type A, remains a major public health problem, occurring endemically in all parts of the world with frequent reports of small and large epidemics. Spread is usually by person to person contact and major outbreaks result most frequently from the faecal contamination of water and food. Subclinical cases are common: the disease has in general a low mortality but patients may at times be incapacitated for many weeks or months. Further general information on transmission experiments to human volunteers and studies in non-human primates may be found in a recent WHO report (Viral Hepatitis: Report of a WHO Scientific Group; Techn. Rep. Ser., 512; 1973).

The discovery of the association between an antigen found in blood and hepatitis type B (serum hepatitis) has prompted efforts to find a similar antigenic marker in patients with hepatitis type A. To date, a specific serum antigen has not been found but examination by immune electron microscopy of faecal extracts prepared from patients with acute hepatitis A yielded interesting results. An antigen was detected by immunodiffusion in faecal extracts from about 50% of patients admitted to hospital in Australia with hepatitis A and in only 2% of control patients (Ferris et al., Lancet, ii, 243; 1970). Antigenic activity was associated with two types of particles measuring 15-25 nm and 40-45 nm in diameter. Animal antisera to this faecal antigen yielded encouraging results but also a number of non-specific reactions, and studies are currently in progress with antisera with improved specificity.

In December 1973, virus-like particles measuring 27 nm in diameter were demonstrated by immune electron microscopy in faecal extracts obtained from 2 out of 4 adult volunteers who were infected by injection or by









Painted panels from the ceiling of the Natural History Museum, London, According to The British Architect and Northern Engineer of June 1878 "the chief idea . . . is that of growth". Left to right: Scots pine Pinus sylvestris; peach Amygdalus persica; cacao Theobroma cacao; English oak Quercus robur.

mouth with the MS-1 strain of hepatitis A virus (Feinstone and colleagues, Science, 182, 1026; 1973). The particles were heavily coated with antibody and they were aggregated by convalescent serum. These particles were not found in faecal specimens before infection, they were only detected at the onset of clinical symptoms, and pre-illness sera did not contain antibodies to the viruslike particles. Faecal filtrates containing these 27 nm virus-like structures were used to examine by electron microscopy several groups of sera for the presence of antibody. All 6 volunteers previously infected with hepatitis A developed serological evidence of infection as judged by aggregation and antibody coating of the 27 nm particles. Similar results were obtained with sera from patients with a number of naturally occurring outbreaks of hepatitis A. Other studies have shown that the buoyant density of the faecal particles in caesium chloride was 1.41 g ml-1 and together with the morphological appearance and known resistance to ether, acid and heaf the data suggest that this particle is a parvovirus (Feinstone and associates, J. Virol., 13, 1412; 1974). Morphologically and antigenically similar particles were also demonstrated in faeces from patients from at least one geographically separated outbreak of hepatitis A (J. Maynard and colleagues, personal communication). The particles identified in Arizona banded, however, in two peaks in caesium chloride at 1.29-1.33 and at 1.39 g ml<sup>-1</sup>. Preliminary data from experimental transmission to marmosets and susceptible chimpanzees have also demonstrated a close association between the faecal particles and hepatitis A.

On the other hand, it is generally recognised that faeces contain a multitude of bacterial, bacteriophage, viral and other particulate antigens. Almeida and her colleagues (Lancet, ii, 748; 1974) examined by immune electron microscopy and by immunodiffusion faecal, extracts and acute and convalescent sera from an outbreak of hepatitis A. Faecal extracts reacted with convalescent sera contained frequent distinctive aggregates having the appearance associated with immune complexes. But particles resembling bacteriophage, particles resembling togavirus and a range of spherical particles were all present in distinct immune complex form. Serological analysis by gel diffusion showed seroconversion to more than one antigen present in the faeces, and seroconversion was also detected to antigens present in a number of control faecal extracts. It was also pointed out that it is known that antibody titres to bacterial, viral and dietary antigens are increased in patients with acute and chronic liver disease and this may account for the multiplicity of immune reactions in viral hepatitis.

The latest results reported by Almeida and colleagues (Lancet, ii, 1083; 1974) after the examination of faecal extracts from one of the infected adult volunteers tested by Feinstone and associates (loc. cit.) confirmed the presence of the 27 nm particles, but at least two small cubic virus-like particles were found in addition. Using the technique of immune electron microscopy in London, extracts of faeces obtained 33 days after infection with the MS-1 strain of hepatitis A were found to contain virus-like structures which varied in their size. Both 'full' and 'empty' particles measuring 22 nm, 27 nm and 30 nm in diameter could be distinguished and they seemed to have a cubic symmetry. Each of these viruslike structures was clearly coated with antibody and the particles were aggregated by convalescent but not by acute serum from hepatitis type A. The fact that this material has also induced hepatitis in 2 out of 6 marmosets suggests that hepatitis A virus is indeed present. But the precise relationship to the disease of the three virus-like particles now described remains uncertain.

The success of immune electron microscopy in elucidating the complex antigenic reactivities of hepatitis B virus may blaze a trail for what seems to be an equally puzzling array of particles present in the faeces, and perhaps the serum, of patients with hepatitis A infection.

## Eclogite model of upper mantle

from Peter J. Smith

THE view that the Earth's upper mantle might be eclogitic in composition was put forward over 60 years ago by Fermor (Rec. Geol. Surv. India, 43, 41; 1913), although since then it has received comparatively little support. According to Green and Ringwood (Phys. Earth Planet, Interiors, 3, 359; 1970), for example, "the weight of geological and geophysical evidence on upper mantle composition favours an overall peridotitic composition", and the arguments against an eclogitic composition were given by Ringwood and Green (Tectonophysics, 3, 383; 1966). During the past decade, Green and Ringwood (see, for example, Earth planet. Sci. Lett., 3, 151; 1967) have given particular attention instead to their "pyrolite" composition, although the validity of this system is by no means universally accepted.

Interest in the eclogite model began

to revive in 1970 when Press (Phys. Earth Planet. Interiors, 3, 3; 1970) used a Monte Carlo procedure to derive a series of Earth models consistent with geophysical data. Specifically, he found that of about 50 acceptable density models, about 30 required densities of at least 3.6 g cm<sup>-1</sup> somewhere in the upper mantle. Both Press and Birch (Phys. Earth Planet. Interiors, 3, 178; 1970) interpreted these high densities to favour almost exclusively an eclogitic composition for the upper mantle, and thus for the low velocity zone, although Birch did add that an iron-rich olivine upper mantle might still be possible. At this point, however, it became clear that any detailed discussion of an eclogitic upper mantle would be seriously hampered by the relative lack of experimental data on eclogites, in particular on their elastic properties.

Some results on the seismic velocities in eclogites have now been provided by Rao et al. (Earth planet. Sci. Lett., 23, 15; 1974), and they show just how involved the situation is. Eclogites from India, Czechoslovakia and Japan all have comparable garnet compositions and mean atomic weights, but there are significant differences in P wave velocities and velocity-pressure gradients. Velocities at 4 kbar, for example, range from 7.43 km s<sup>-1</sup> for the Indian eclogites, through 8.05 km s<sup>-1</sup> for those from Japan, to 8.35 km s<sup>-1</sup> for the Czechoslovakian samples. For the same location order, average velocitypressure gradients are, respectively, 0.08, 0.058 and 0.036 km s<sup>-1</sup> kbar<sup>-1</sup>.

The reason for these variations is that the apparent distribution constant (K) of Fe-Mg between coexisting garnets and pyroxenes varies from one set of eclogites to the other, indicating that the conditions of temperature and pressure under which they formed were different. In fact, Rao and his colleagues are able to demonstrate a linear relationship between P wave velocity and K. But details apart, the general point to be made is that if the eclogite model is now to be considered seriously, much more experimental work will be required to elucidate the complexities of eclogite systems—as much, perhaps, as has been done on the more popular pyrolite systems.

#### Erratum

In the article "The Linear Differentiation of Chromosomes" (Nature, 252, 95; 1974) the statement was made that "the many current attempts to explain banding reflect our lack of knowledge of how 1 cm of DNA can be packed with protein into a 1 mm transverse band". 1 cm of DNA is in fact packed into a 1  $\mu$ m transverse band.

### review article

### Congenitally abnormal vision in Siamese cats

R. W. Guillery, V. A Casagrande & M. D. Oberdorfer

In albino mammals, the absence of pigmentation in the eye is associated with abnormalities of the visual pathway and these result in a scrambled input to the visual centres of the brain. Recent research on Siamese cats, in which more than one pattern of abnormality has been found, shows that there are several ways in which the cortex can deal with the abnormal input, and is beginning to define the rules according to which the cortex selects its inputs.

THE sensory centres of the brain carry an orderly representation of the environment as shown by many topographically organised sensory 'maps'. While it seems likely that such orderly representations are essential for the proper functioning of sensory mechanisms, it is difficult to test this rather general view, because one cannot easily challenge sensory mechanisms by providing them with a scrambled input. Recently, however, the discovery that some mammals have congenitally abnormal visual pathways has provided an opportunity for testing how cerebral mechanisms can respond to disordered inputs, and studies of the abnormal pathways have begun to show some of the rules on the basis of which cerebral representations of the outside world can be established.

Mammals with abnormally low amounts of pigment in the retinal pigment epithelium have abnormal pathways linking the eyes to the brain<sup>1-8</sup>. Generally, the abnormality involves nerve fibres that arise in the temporal parts of the retina. Whereas in normal animals all such fibres would pass to the cerebral hemispheres without crossing (Fig. 1), in the abnormal animals some of the temporal fibres cross the midline (Fig. 2a).

#### Retino-geniculate connections in Siamese cats

The precise pattern of the abnormal projection has been worked out in most detail for the lateral geniculate nucleus of Siamese cats<sup>9-11</sup>, and Fig. 2(a) summarises the results obtained by anatomical and electrophysiological methods<sup>9</sup>. The basic pattern of the abnormality is simple. Some of the retino-geniculate axons go to the wrong side of the brain, but the geniculate locus of all axon terminals relates to the retinal locus of their cells of origin in a normal mapping. Thus, in Fig. 2a, segments 10\*, 11\* and 12\* of the left retina are shown passing to the right lateral geniculate nucleus instead of the left. Within the right nucleus they terminate in the appropriate cell layers (shown as layer A1 in Fig. 2) and the axons that arise in segment 10\* end medially, while those arising in segment 12\* end laterally, as is normal. It can be seen that segments 5\*, 6\* and 7\* from the left eye, which would normally map opposite segments 5+, 6+ and 7+ from the right eye, in Siamese cats map opposite segments 12\*, 11\* and 10\*, respectively, from the left eye.

The distribution of the abnormal fibres does not vary greatly in Siamese cats. There is always a lateral, normally connected segment of lamina A1 (3<sup>+</sup> and 4<sup>+</sup> in Fig. 2a, the 'lateral normal segment') and next to it is a large

abnormally connected segment, the 'lateral abnormal segment'. The border between the two (arrow in Fig. 2a) generally receives from retina that lies 15° to 20° from the line of decussation. Most of the cats that have been examined also show a small 'medial normal segment' of lamina A1 (8<sup>+</sup> in Fig. 2a), but the size of this segment varies somewhat between animals, and it may possibly not occur at all in some Siamese cats (see below).

Fig. 2a shows that the lateral geniculate nucleus receives an abnormal representation of the visual field. It seems as though there are geniculate mechanisms that sort axons in terms of retinal topographies, but that there are no mechanisms for correcting the nonsense appearing in terms of orderly visual field representations. We shall see that this is in striking contrast to the situation in the visual cortex.

There are three major consequences of the simple retinogeniculate misrouting, and we shall refer to them as a 'disruption', an 'inversion' and a 'non-correspondence'. The first term refers only to the fact that within one geniculate layer the visual field segments are represented in an interrupted manner. An inversion occurs when a portion of the visual field representation is a reversal of the normal, so that one finds an ascending sequence in one part of layer A1 (10\*, 11\*, 12\* in Fig. 2a) and descending sequence in another portion of the same layer (4+, 3+). An inversion is a special case of a disruption, and each involves only a single geniculate layer. A non-correspondence, which is any mismatching of two adjacent. layers, is necessarily an interlaminar phenomenon. It is possible to produce a non-correspondence without a disruption and to produce a disruption without a reversal, and thus, it is useful to consider the three as distinct abnormalities.

### The geniculo-cortical pathways in Siamese cats

Figure 1 shows that in a normal cat the visual cortex receives an orderly, binocular representation of the contralateral visual field. Hubel and Wiesel<sup>12</sup> have established that most neurones in the visual cortex receive inputs from both sets of geniculate layers and can be activated through either eye. Such a balanced, binocular geniculo-cortical projection would clearly lead to serious problems in Siamese cats, since cortical activity would provide an ambiguous representation of the environment. Figure 2b shows that if the geniculo-cortical pathways in Siamese cats were arranged as in normal cats, some cortical celproups would be activated by two quite different parts of the visual field. Further, a movement from left to right across the visual field would be represented ambiguously

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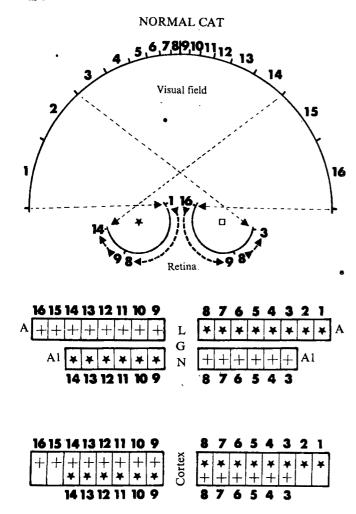


Fig. 1 The normal retino-geniculo-cortical projections in a cat. The representations of the visual field within the visual centres are shown as numbered sequences. Those coming through the right eye are marked by plus signs, and those coming through the left eye by stars.

within the cortex, being in one direction for segments 5\*, 6\* and 7\*, and in the opposite direction for segments 10\*, 11\* and 12\*.

Let us first consider the non-correspondence produced by the abnormality in terms of information available about normal cats. The visual cortex of normal cats can be made to receive noncorrespondent inputs by quite simple manipulations. When normal cats are reared with a surgically induced squint, the inputs to adjacent geniculate layers are made non-correspondent and single cortical cells no longer receive binocularly matching stimuli<sup>13</sup>. The geniculocortical pathway in such cats becomes modified so that individual cortical cells receive a monocular input. Cells receiving from each eye tend to be grouped together in distinct cortical columns. Within the cortex, instead of a single binocular representation of the visual field, two separate monocular representations are formed, and these are arranged as a mosaic of interdigitating monocular columns. A similar result has been obtained in kittens reared with alternating occlusion of the eyes<sup>13</sup>. Again, cortical cells were exposed either to right eye or left eye stimulation; since they were never exposed to correspondent binocular inputs, no binocular cortical columns were formed.

On the basis of these observations on non-correspondent geniculate inputs one might expect a similar result in Siamese cats. Considering first a Siamese cat with no strabismus, one might expect the situation illustrated in Fig. 2b, which shows a hypothetical, not an actual cortical

pattern. Binocular columns are shown in the cortical regions that receive from the normal segments of the lateral geniculate nucleus (3 4, 8 in Fig. 2b) and a mosaic of monocular columns is shown for the abnormal segments (5\*, 6\*, 7\*, 10\*, 11\*, 12\*). A cat with a significant strabismus, and this is quite common in Siamese cats, might be expected to show two independent sets of monocular columns for the whole visual field representation. The Siamese cats that have been studied so far, however, have shown a strikingly different cortical pattern.

### Cortical suppression of the temporal visual field

The pattern of neuronal activity that we have found in the visual cortex of most Siamese cats<sup>14</sup> is shown schematically in Fig. 3a. The input coming through lamina A from the contralateral retina (1\* to 8\*) appears to be quite normal, but it is difficult to record much cortical activity corresponding to an input from lamina A1. Segments 8<sup>+</sup>, 10\* to 12\*, 4<sup>+</sup> and 3<sup>+</sup> are, therefore, shown as thinner figures and smaller symbols in Fig. 3a. It seems that the visual cortex in these animals is looking at the whole visual field through the A laminae on each side, and that all of the inputs coming through the A1 laminae, which contain an inversion, are suppressed.

The cortical activity suggests that these Siamese cats have a normal field of view when they are using both eyes, but when they are only using one eye the effective field of view is limited to the parts of the visual field seen by the nasal retina. That is, the cat sees on the side of the open eye, but does not see across the midline at all.

Elekessy et al.<sup>15</sup> have used behavioural methods to study the visual fields of Siamese cats. They showed, independently, almost the exact limitation of the visual fields that our results led us to expect. Whereas normal cats have about 135° of visual field in each eye<sup>16,17</sup> (Fig. 1), their Siamese cats had only about 90° of visual field extending from close to the midline to the temporal periphery (1 to 8 for the left eye in Fig. 1).

It is clear that these Siamese cats do not develop a binocular mosaic of independent monocular inputs. Instead, there is massive suppression of the inverted segment (10\*, 11\*, 12\*, in Fig. 3a) and even the segments of the visual projection that are normal (3\*, 4\*, 8\*, in Fig. 3a) do not establish a demonstrable, cortical field. The suppression in these normally connected segments shows that lateral interaction must play a part in the development of the geniculo-cortical pathways. Segments 3\* and 4\* in Figs

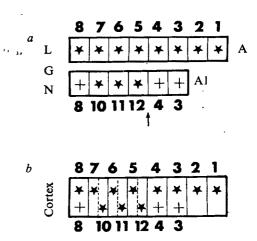


Fig. 2 The abnormal geniculo-cortical pathway to the right hemisphere in Siamese cats. a, Abnormal representation of the visual fields found in the right lateral geniculate nucleus of Siamese cats. b, Cortical patterns that might be expected if the geniculo-cortical pathways were normal (see

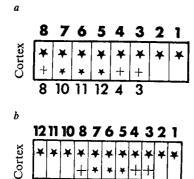


Fig. 3 a, Cortical representations of the visual field found in 'Midwestern' Siamese cats. b, Cortical representations of the visual field found in 'Boston' Siamese cats. The lighter numbers and smaller symbols represent a weak cortical input.

2 and 3a can only be regarded as abnormal because they form a part of a sequence that contains an inversion. It seems that the cortex does not accept inverted representations of the visual field, even though it can accept non-correspondent ones.

### Correction of the inverted representation

While the cortical pattern shown in Fig. 3a is found in some Siamese cats, other Siamese cats show a second pattern. This has been demonstrated electrophysiologically by Hubel and Wiesel<sup>11</sup> and by our own anatomical evidence<sup>14</sup>. The most striking feature of this second pattern is the reversal of the geniculo-cortical projection of segments 10\*, 11\* and 12\* (see upper row in Fig. 3b) and the insertion of these segments into cortex that normally receives from close to the zero vertical meridian. The cortical input from these abnormal geniculate segments to the expected cortical locus (opposite 5\*, 6\* and 7\*) is correspondingly reduced. The total amount of the visual cortex is not increased in these cats, but about 135° of visual field is represented in the cortex instead of the normal 90°.

The sequence of the visual field representations shown in bold figures is normal, except for the hiatus between 8 and 10, and the size of this hiatus on the right corresponds to the size of the medial normal geniculate segment on the left (9\* and 9\*). Thus, the modified geniculocortical projection in these cats has corrected the reversal, but appears to have left a small disruption. Any cat in which the medial normal segment was small or absent would show a virtually complete recreation of the normal sequence of visual field segments, and it is possible that the size of the medial normal segment is critical in determining whether the cortical pattern shown in Fig. 3a or that shown in Fig. 3b will be formed. If normal cortical function depends upon an uninterrupted sequential ordering of the visual field representations in the cortex, a cat with a large medial normal segment, which could establish a normal sequence only by suppressing the input from layer A1, would have to use the solution shown in Fig. 3a, while a cat with a small medial normal segment might use the more elegant solution illustrated by Fig. 3b.

We have called these two types of cortical pattern the Midwestern (Fig. 3a) and the Boston pattern (Fig. 3b)<sup>14</sup>. We do not know whether the formation of one or the other is related to the size of the medial normal segment. In fact, we know nothing of the mechanisms controlling the development of these pathways. The problem has been discussed by Gaze and Keating<sup>18</sup>, and it is relevant that the solution they propose for the formation of the Boston pattern also assumes an absence of the medial normal segment.

No one has yet tested the visual fields of a Boston cat. From the cortical records that have been obtained", however, one can reasonably expect that Boston cats have a normal 135° field of view. The visual field test may therefore prove to be useful for further studies of the Siamese abnormality, since it is relatively simple and can be made without any injury to the cat. Some such test for distinguishing Boston from Midwestern cats will be needed for studies of the inheritance patterns of the abnormalities and for further developmental studies.

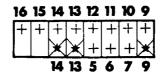
### Effects of monocular deprivation in Siamese cats

The effect of the inversion in the lateral geniculate nucleus can be tested by raising Siamese cats with one eye sutured. In Midwestern cats monocular deprivation will have the effect shown by crosses in Fig. 4. On both sides the inversion of the visual field representation within lamina A1 will be abolished. Further, the parts of lamina A1 receiving visual input will form a continuous, though partial representation of the visual field on one side (5<sup>+</sup>, 6<sup>+</sup>, 7<sup>+</sup>), and a disrupted representation on the other (3<sup>+</sup>, 4<sup>+</sup>, 8<sup>+</sup>). This disruption should be distinguished from the type of disruption that occurs, for example, in Fig. 3bbetween segments 8 and 10. In Fig. 3b there are no geniculate segments between 8 and 10, and we regard this as a 'strong' disruption. In Fig. 4 the amount of geniculate nucleus between segments 4 and 8 is appropriate for the intervening visual field segments, and we call this a 'weak' disruption.

In normal cats monocular deprivation upsets a balanced binocular competition and leads to a dramatic reduction in the cortical input from the deprived eye<sup>17,19,20</sup>. In Siamese cats monocular deprivation will upset this balance in geniculate segments 3, 4, 8 (Fig. 2a), but will not affect it in the rest of the nucleus.

Three Siamese cats that were raised with one eye sutured have been studied electrophysiologically<sup>14</sup>. All three cats showed a Midwestern pattern, but in each, stimulation of the temporal retina activated cortical cells more effectively than in normal Siamese cats: although the input from lamina A was still dominant throughout the cortex in these three kittens, all parts of lamina A1, except for the normal deprived segments (9\*, 13\*, 14\*) showed this increased effectiveness. These changes occurred within the abnormal segments even though the balance of the translaminar interaction had not been altered at all: it was the sequence of the representation within lamina A1 itself that had been altered by the deprivation.

More recently we have raised three Siamese kittens with one eye sutured from postnatal day 9 until they were 6-9 months old. We then tested their visual fields behaviourally, using essentially the same methods as Elekessy et al. (see ref. 17 for details). Visual responses were tested along guidelines at 15° intervals and we found that all three kittens had about 135° of visual field for the nondeprived eye (Table 1 and Fig. 5). That is, the visual fields of the nondeprived eye were like those of a normal cat, as would be expected from our earlier electrophysiological observations. Suturing one eye had increased the effective visual field of the other.



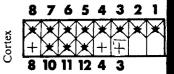


Fig. 4 Schema to show the expected effects of monocular visual deprivation in a Midwestern Siamese cat. Segments that are deprived by suturing the left eye shortly after birth are shown by large crosses.

Table 1 Test scores for	guidelines at 15° intervals,	(% of correct responses	s: 50 trials for each score)*

	**	_		20groon	from midlin				
	Eye	· On	side of open	eye		On side of	closed eye		
Animal	tested	105	90	75 to 0	15	30 ·	45	60	Notes
668	R	··· 0	28	80-100	51	0	0	0	5
	L	_0	37	80-100	26	0	0	0	
OM	R	<b>1</b> 1	73	90-100	50	6	0	0	
	L	10	55	90-100	92	14	0	0	
OF	R	21	84	100	33	0	0	0 .	
	. <b>L</b>	7	69	90-100	73	24	0	0	
636	R	0	56	90-100	95	98	54	24	Left eye sutured
637	L	15	86	90–100	100	98	36	0	Right eye sutured
635	L	17	85	90-100	96	85	19	0	Right eye sutured
635	R & L	0	43	100	100	83	83	0	Sutures removed at 7 months

<sup>\*</sup> Final scores are corrected as follows: Where blank scores (B) were greater than guideline scores (G) (see ref. 17 for definition) the corrected score was G-B 100-B

We regard all three kittens as Midwestern: the retrograde degeneration produced by a cortical lesion has demonstrated this for one of the kittens (see ref. 14 for method). The other two are still living, but since the overwhelming majority of the cats we have obtained locally have been Midwestern cats, and since the two parents (OM and OF) and one unrelated control (668) have shown a limitation of the monocular fields that is not normal (Table 1 and Fig. 5), the conclusion that we were dealing with Midwestern cats seems justified, even though there is some spread of visual responses across the midline in the two parents. This spread may represent uncontrolled eye movements or may, perhaps, indicate some vision in the temporal retina. Since the amount of the visual sparing is distinctly less than would be expected in Boston cats and since there are no grounds for expecting such a limited sparing in Midwestern cats, we favour the former view.

It remains to be pointed out that in these three kittens we were testing cortical, not tectal functions: it is known that the tectal visual fields demonstrable behaviourally do not cross the midline (ref. 16 and M. S. Sherman, unpublished), and the behavioural results matched the activity recorded in cortical nerve cells of comparable cats<sup>14</sup>.

The visual cortex in the monocularly deprived Siamese cats was, therefore, able to produce accurate control of movement in spite of the fact that within one area of cortex two different parts of the visual field were represented in reverse order (10<sup>+</sup>, 11<sup>+</sup>, 12<sup>+</sup>; 7<sup>+</sup>, 6<sup>+</sup>, 5<sup>+</sup>). The non-correspondence was dealt with successfully, even though the two representations were opposed in direction, and presumably a mosaic of alternating monocular columns was established in the cortex. The critical difference between the normal and the monocularly deprived Siamese cats is the lack of any inversion within a single geniculate layer. Thus, the cortex must monitor its geniculate inputs in terms of the sequences coming from individual layers. Non-correspondences can be accepted, even when they involve complete reversals, but inversions within one geniculate layer are unacceptable. The weak form of the disruption occurring between segments 8+ and 5<sup>+</sup> in Fig. 4 seems not to prevent the formation of cortical connections, but we have seen that there is some indirect evidence that the strong form of the disruption occurring between segments 8\* and 10\* in Fig. 3b may be unacceptable.

When the tests summarised above were completed, the sutured eye was opened and the testing continued with this eye only. To date, we have found no signs of any vision in the deprived eye. This is in marked contrast to a normal

cat: when a normal cat is raised with the left eye sutured, the visual functions are spared in the right monocular geniculo-cortical segments (1 and 2 in Fig. 1) within which the deprived pathway has not had to compete with the normally innervated one. Even though segments 1 to 4 are

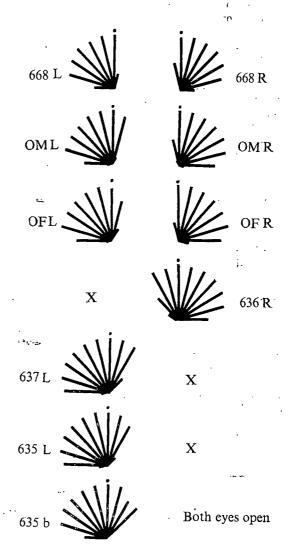


Fig. 5 Schematic representation of corrected guideline scores from table 1. The midline is indicated by a dot. X shows the sutured eye. 635b shows visual fields in a cat that was raised with the right eye sutured and that was subsequently tested with both eyes open.

connected into the retinocortical pathways in the same way in normal and Siamese cats, segments 1 and 2 react to lid suture in quite different ways in the two sorts of cat.

This is a puzzling observation and there is, at present, no direct evidence to explain it. We suggest that the difference must be produced by the abnormality in segments 10\*, 11\* and 12\* (Fig. 4). If an adult cat is no longer able to establish a mosaic of alternating monocular columns, then the cortex of our monocularly deprived cats had no way to separate the lamina A inputs from the lamina A1 inputs when the deprived eye was opened. The total new input pattern contained an inversion and this is why the whole sequence was suppressed at cortical levels.

It would seem then that the development of cortical 'maps' depends upon the coherence of the sensory representation that can be established in the cortex. A noncorrespondence can be turned into two independent coherent cortical representations in an infant, but probably not in an adult. Incoherence, whether produced by a strong disruption, by an inversion, or by a fusion of non-correspondent inputs, leads to blocked development of a cortical representation. In contrast to this, geniculate representations, even when they are incoherent in terms of the visual field, are successfully established on the basis of retinal locus.

We have shown that the abnormality found in albino mammals can be used to study the mechanisms by which orderly visual field representations may be established. So far we have only considered problems that arise in the geniculo-striate system of Siamese cats. Other parts of the visual pathway are also abnormal in these cats 10,21,22, however, and it may prove that they develop according to different rules. Further, the precise pattern of the abnormality differs in other species. Albino ferrets show a very large abnormality and have practically no uncrossed retinogeniculate axons'. Abnormal pathways have been found in several genotypes of mink<sup>8</sup> and the size of the abnormality, which is related to the amount of pigment in the retina, varies considerably. Thus there is a variety of models on which one can test hypotheses about the visual pathways.

So far our studies of Siamese cats have shown that in at least one sensory system the cortex monitors the sequence of its sensory inputs, and that there are definable rules according to which disrupted inputs can be rejected.

We thank Dr J. H. Kaas who collaborated in many experiments and Mrs E. Langer and Mrs B. Yelk who helped with the preparation of materials. The work was supported by grants from the Public Health Service, National Institutes of Health.

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## articles

### Relationships between sealevels, <sup>18</sup>O variations and orbital perturbations, during the past 250,000 years

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Sealevel changes over the past 250,000 yr, determined from dated coral reefs on rising islands, are synchronous with precessional changes in solar radiation when orbital eccentricity is large. Oxygen isotope variations in deepsea cores correspond in timing but not in amplitude, reflecting changing isotopic composition of Pleistocene icecaps.

WORLD climates during Quaternary times were influenced by two primary factors. First, there have been variations in the geographical and seasonal distribution of solar radiation, which resulted from perturbations in the Earth's orbital motion. Second, there was the role of the oceans in transferring heat from low to high latitudes, thus influencing strongly the periodic growth of the Laurentide and Fennoscandian Pleistocene icecaps.

The analysis by Milankovitch<sup>1</sup> of orbital perturbations

and variations of radiation has been supported by several workers<sup>2-4</sup>. This astronomical theory of climatic change has, however, been criticised<sup>5-8</sup>, principally because changes of radiation are themselves very much smaller in magnitude than the observed Quaternary temperature changes. Advocates of the astronomical theory suggest that icecap growth and decay may both be self-amplifying processes, under certain conditions, and that triggering from one state to the other is effected by the changing distribution of solar radiation<sup>8,10</sup>. The case for the astronomical theory rests essentially on a correspondence between insolation curves and well dated records of Quaternary temperatures or ice volumes. Until recently, oxygen isotope and micropalaeontologic studies of deep-sea cores provided the best basis for such arguments. The statistical methods for correlation between the core records and insolation curves have, however, been disputed11,12; and the time scales for the cores are also disputed, with consequent disagreements about correlations13-17

Results which lend support to the astronomical hypothesis come from flights of coral reef terraces that occur in rapidly rising areas, and which have been dated by <sup>230</sup>Th/<sup>234</sup>U. The important results are from Barbados<sup>4,18,16</sup> New Guinea<sup>20-22</sup>, and the Ryukyu Islands<sup>23,24</sup>. Age estimates for Upper Quaternary sealevel maxima agree closely between the areas22, and from New Guinea the record is especially good. The recurrence of transgression-regression cycles with a period of about 20,000 yr has been claimed to reflect the precession effect upon Pleistocene climates<sup>4,20</sup>. and the New Guinea sealevel curve correlates strongly with isolation changes over the last 0.23 Myr (ref. 25). Figure 1 shows sea level level changes during that period, together with  $\delta^{18}O$  records from deep-sea cores from the Caribbean26, and the equatorial Pacific27. Orbital perturbation effects for the same period are also shown. To allay previous disputes about time scales and palaeosealevels, these curves will be discussed more fully.

The sealevel curve is based on the series of transgressionregression cycles identified from successions of offlapping coral reefs, mainly in New Guinea, which have been dated precisely by <sup>230</sup>Th/<sup>234</sup>U with supporting <sup>14</sup>C data<sup>20-22</sup>. The estimated magnitudes of sealevel oscillations, and the basis for separating tectonic and eustatic effects have been explained20-22. The curve for the past 0.14 Myr is taken directly from the most recent results, and this is highly in agreement with the less complete Barbados record<sup>22</sup>. The curve before 0.14 Myr is an updated version of that published earlier20,21, based on precise levelling and field observations which I made in 1973, and which will be published elsewhere. The only significant difference between the new and the earlier curve is that the regression between 0.17 Myr and 0.14 Myr achieved a level of at least -100 mand possibly lower (as against the previous estimate of -55 m or lower). The  $\delta^{18}$ O curves shown are from Caribbean core P6304-8 (ref. 26) and equatorial Pacific core V28-838 (ref. 27); both are based on the pelagic foram, Globigerinoides sacculifera. The time scale for the cores was given by Shackleton and Opdyke<sup>27</sup>, and was based on clear identification of the Brunhes-Matuyama magnetic boundary, and the assumption of constant sedimentation rate. This time scale is very similar to that proposed by Broecker et al.<sup>14,17</sup>, and is about 25% longer than that proposed by Emiliani<sup>16,26</sup>. The critical issue of a time scale for the cores is subject to a significant scatter in reported U-series age determinations, and opinions diverge about core dating by this method<sup>13-15</sup>. To find additional support for the shorter time scale, Emiliani et al. argued that the generalised palaeotemperature curve shows good peak correspondence with the 65°N isolation curve3, and with dated raised beaches16. Many statistical arguments of this kind are, however, incorrectly founded25, and there is no significant correspondence between the New Guinea sealevel curve and Emiliani's generalised palaeotemperature curve<sup>25</sup>. I concur<sup>25</sup>, however, with the results of Kemp and Eger<sup>28</sup>, which support a significant correspondence between Emiliani's curves and the high latitude insolation curve. The correlation is not as strong as those evident in Fig. 1, however, and this presentation of the most accurate sealevel and  $\delta^{18}$ O results to date is taken as resolving the time scale argument for deep-sea cores in favour of the Broecker-Shackleton-Opdyke scale.

The most important features of Fig. 1 are first, the large number of peak-to-peak correspondences, especially between the sealevel curve and the precession-dominated curve of variations of Earth-Sun distance; second, the small degree of comparability between sealevel and δ18O curves in terms of relative amplitudes of oscillations; and third, the greater excursions of δ<sup>18</sup>O shown in the Caribbean core relative to the Pacific core (true also for other Atlantic and Caribbean cores<sup>29</sup>). These facts have extensive implications for ice age mechanisms. The very strong correspondence between fluctuations in sealevel and variations of the mean solar distance shows that the growth and decay of high latitude icesheets is influenced very strongly by insolation at low latitudes (that is because precession effects dominate insolation curves at low latitudes; obliquity dominates at high latitudes).

Control of Quaternary climatic fluctuations by variations in insolation at low latitudes was suggested by Broecker<sup>4,30</sup>: it is to be expected on meteorologic grounds<sup>31</sup>; and the principle is reinforced by the finding<sup>32</sup> that radiation input at high latitudes is quite inadequate to have caused Late Wisconsin deglaciation in the time available.

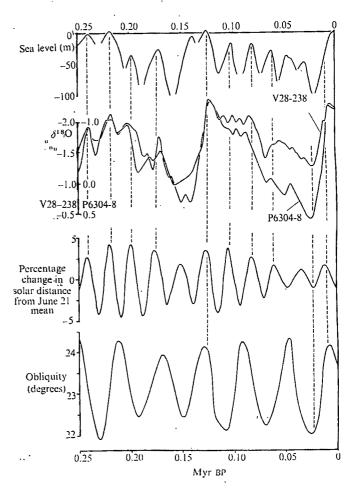


Fig. 1 Sea levels, δ<sup>18</sup>O variations, and orbital perturbation effects for the last 250,000 yr. Emiliani's results<sup>26</sup> for core P6304-8 are plotted on the time scale of Shackleton and Opdyke<sup>27</sup> (see text). P6304-8, Atlantic core<sup>26</sup>; V28-238, Pacific core<sup>27</sup>.

The Laurentide and Fennoscandian ice sheets—over 85% of the Pleistocene ice excess<sup>8</sup>—were nourished principally from the North Atlantic<sup>8,33</sup>, into which the Gulf Stream-North Atlantic current is the major conveyor of heat.

The relative amplitude differences between sealevel and  $\delta^{\iota s} \mathrm{O}$  curves are important, as the latter have often been argued to be directly indicative of the former<sup>34-36</sup>. The greatest discrepancy is between 0.115 Myr and 0.08 Myr, when two sealevel oscillations of about 50 m occurred: from the δ18O record, Shackleton27 has inferred approximately constant temperatures and sea levels, fluctuating within a 15 m range. From 0.075 Myr to 0.015 Myr the amplitudes of sealevel oscillations are again underestimated using  $\delta^{18}$ O. There is also an important shift in the mean  $\delta^{18}$ O level. For the Pacific core,  $\delta^{18}$ O is about +0.25%with respect to the mean Holocene level, from 0.115-0.08 Myr; from 0.075-0.015 Myr the shift is about +0.75%. The sealevel curve shows that considerable icecaps waxed and waned from 0.12-0.08 Myr BP, thus δ18O evidence suggests either that mean temperatures were considerably higher during this period than in the next 0.065 Myr, or that the icesheets formed around 0.11 Myr BP and 0.09 Myr BP were not as relatively poor in <sup>18</sup>O as those from 0.075 Myr BP to 0.015 Myr BP. The latter hypothesis may be simply evaluated. Mean sealevel between 0.115 Myr BP and 0.08 Myr BP (about 1% of mean ocean depth). Mean ocean enrichment of  $\delta^{18}$ O was  $\approx 0.25\%$  and therefore ice formed should show  $\delta^{18}$ O impoverishment by -25%. For the period 0.075-0.015 Myr BP was about -75 m, and with mean ocean  $\delta^{18}0 \simeq 0.75$ , ice formed in that period would show  $\delta^{18}O \simeq -37\%$ . Such a  $\delta^{18}O$  shift pattern is evident in the Greenland ice core<sup>37</sup>. These estimates indicate the general result of ocean volume change, and are not intended to negate palaeotemperature inferences (the effect of which the uptake of <sup>18</sup>O by marine organisms, has been well demonstrated<sup>38</sup>).

If the amplitudes of oscillations in the  $\delta^{18}$ O curves are low compared with the sealevel curve, that can be explained partly by mixing in the sea-bottom sediment<sup>27</sup>, and by the vertical migration of pelagic forams in response to glacial-interglacial salinity changes<sup>39</sup>. It is also partly explicable in terms of the  $\delta^{18}$ O distribution in precipitation over the icecaps. Precipitation at the centre and northern sector of the Laurentide icecap is likely to have been impoverished by about 20%, relative to the southern margin<sup>40</sup>. During icecap retreat, the water returned to the ocean was mainly from the periphery and the southern sector until the retreat was well established, whilst snow continued to fall over the icecap centre. This snow may have been more than 10% depleted in 18O, with respect to the meltwater. Although deep ice flow would move highly <sup>18</sup>O-deficient ice from the centre outwards, the rate would be slow compared with wastage of the flanks<sup>40,41</sup>. Thus the mean 818O level should rise during icecap retreat (up to a certain stage of icecap diminution), and the ocean δ<sup>18</sup>O swing is therefore reduced relative to the sealevel swing. Estimation of the magnitude of this 'fractionation' effect requires careful modelling.

There are two possible explanations for the apparent shift about 0.08 Myr ago in the mean δ18O value of icecapnourishing snow. A change of climate may have occurred, so that the moisture-bearing airmasses which originated over warmer oceans before 0.08 Myr BP, originated over cooler oceans subsequently. Alternatively, the icecaps around 0.112 Myr BP and 0.095 Myr BP may have been nearly as extensive at the later (Main Wisconsin) sheets, but only about half as thick. These alternatives are deduced from the pattern of  $\delta^{18}$ O values over high northern latitudes, reported by Dansgaard and Tauber40. The ocean temperature effect is illustrated by the  $\delta^{18}O$  difference between precipitation over water fed by the Gulf Stream at 60°N in the Norwegian Sea (-7%) and at the same latitude in the Labrador Basin (about -15%). The mean surface water temperature difference is about 6° C. The altitudinal effect is indicated by an impoverishment of up to 20% in the precipitation over central Greenland, relative to the coasts (a height difference of about 2.5 km).

The  $\delta^{18}$ O shift of about -10% around 0.08-0.07 Myr BP in the ice core from Camp Century, Greenland37, suggests that there was a simultaneous alteration of high latitude climate. This is because Greenland ice is very unlikely to have been 1 km thicker than at present during main Wisconsin times, for dynamical reasons (1 km is approximately the additional elevation needed to produce the observed  $\delta^{18}$ O shift by the altitude effect). The altitude effect cannot be ruled out for the inferred Laurentide and Fennoscandian icecaps of around 112 Myr BP and 95 Myr BP, as these were not dominated by an ocean boundary; however, a climatic change influencing Greenland would have affected these also. The ocean temperature effect on δ<sup>18</sup>O levels in snowfall seems to be implicated. The warmer sources of ice-nourishing precipitation 0.12-0.08 Myr BP were either the northern Atlantic or the Arctic Oceans. For the  $\delta^{18}$ O effect to have stemmed from the Arctic, the Arctic Ocean must have been open water, a condition likely to be stable42 even with a moderate ice cover on the encircling land masses. Open water in the Arctic, suggested by Ewing and Donn<sup>43,44</sup> as a likely factor in initiating glaciation, would lead to mean Arctic surface water temperatures up to 9°-C higher<sup>42</sup>. Arctic deep-sea cores indicate an ice cover on the sea over the last 70 Myr (refs 45 and 46), but interpretations differ for the core record before that time. The evidence from core T3-67/11 is that foram productivity was much greater from about 0.13-0.07 Myr BP than in either the preceding or succeeding 0.07 Myr (ref. 45). Such heightened productivity is consistent with open water in the Arctic45,46. Detailed analysis of further cores is necessary. It may be that the record from the Norwegian Sea, which Kellog<sup>47</sup> amply demonstrated to have been considerably more ice-bound than at present during the last 0.12 Myr indicates sufficiently that the Arctic is unlikely to have been ice free. The conditions of the ocean surface in the northern Atlantic must therefore have altered substantially between 0.08 Myr and 0.07 Myr ago, with probably a fall of temperature and perhaps a major expansion of ice cover over the sea.

Rather rapid alterations of regional climate, occurring well after glaciation is established, may thus be responsible for important differences between  $\delta^{18}O$  and sealevel records for the last 250,000 yr. Within glacial epochs, icecaps oscillated in size with about a 20,000-yr period. When orbital eccentricity is large, icecap growth and decay is synchronous with precession-induced variations of mean solar distance at the summer solstice. These facts must be incorporated in any adequate theory of ice ages.

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### Statistical geometrical approach to random packing density of equal spheres

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A statistical geometrical argument is presented which could, as the basis of a more refined calculation, help to give a statistical geometrical answer to the question of what random packing is.

'WHAT is random packing?' That question has been much discussed since random packings of spheres were first proposed as models of simple liquid structure1-6. Experimental determinations of the maximum packing density agree on a value of about  $0.636 \pm 0.001$  (refs 7-16), but there is still no satisfactory statistical geometrical theory to explain this value, though a rough explanation based on coordination number distribution has been given by one of us17. Moreover, the lower limit of stability of a random packing occurs at a density of about 0.60 (refs 9-13); no explanation of this 'random loose packing' figure exists. We present here a statistical geometrical argument which derives a packing density of 0.6357 in excellent agreement with the recognised experimental values. We also obtain a lower stability bound at a density of 0.6099, which we suggest corresponds to the random loose-packing.

### The statistical argument

Our argument is threefold:

(1) As a consequence of a stability criterion, the average coordination number in the packing should be 6.0.

(2) As first pointed out by Bernal<sup>5</sup>, the random packing can be thought of as a collection of tetrahedral aggregates of spheres. We calculate the most probable shape of the characteristic tetrahedron.

(3) We then deduce the overall packing density from that of the most probable tetrahedron.

### Average coordination number

For a given sphere to be stable against displacement in a given direction, it must be supported by three spheres in that direction, the group of four spheres forming a tetrahedron. The same stability criterion must also apply for the opposite direction, implying thus a coordination number average of 6. This proposition was examined by Heppes<sup>18</sup> for a serially-deposited packing in which no rearrangements are permitted. Bennett19 later argued that the same should be true of a packing further densified by shaking or otherwise. Figure 1 is a plot of the average coordination number against radial distance for the full Finney 7934 centre close-packed model<sup>20</sup> and shows an average of 6 (allowing for experimental errors in the sphere coordinates). Each point is the average value of 500 to 700 central spheres and full curve is the data line of Mason<sup>21</sup>. A recent study22 on a simulated very slow settling of rigid equal spheres into a randomly packed bed gives also an average coordination number of 6.0, with an overall packing density of

#### The most probable tetrahedron

Consider a tetrahedron formed by an arbitrary sphere and three supporting spheres. We postulate that the three supporting spheres supporting a central sphere are oriented according to a probability that is proportional to that projected surface area of the spherical envelope at R = 1.0 diameters which can be seen from a long distance from the supported sphere. We ignore the possible restricting effect of the neighbours on the configuration of the three supporting spheres.

In the coordinate system shown in Fig. 2a, we calculate the expected z coordinate of a first supporting sphere. The elementary annular surface area of the spherical envelope at  $z = z_1$ which can be seen from distant z direction is

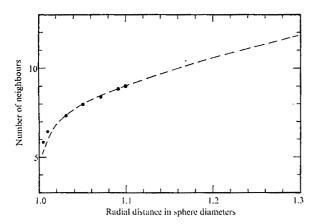


Fig. 1 The average number of neighbouring spheres within the radial distance R in units of the sphere diameter.  $\bullet$ , this experiment; --, ref. 21.

$$dS = 2\pi r_1 \cdot d\varphi_1 \sin \varphi_1 = 2\pi z_1 dz_1$$

where the subscript 1 denotes the first supporting sphere. The probability that the z coordinate of the first supporting sphere lies between  $z_1$  and  $z_1 + dz_1$  becomes

$$f(z_1) dz_1 = (2\pi z_1 dz_1)/(\int_0^1 2\pi z_1 dz_1) = 2z_1 dz_1$$
 (1)

where  $f(z_1)$  is the probability density function, and the denominator in the middle term of equation (1) expresses the total area available for the first sphere. From equation (1) the expected value of  $z_1$  becomes

$$\bar{z}_1 = \int_0^1 z_1 \cdot f(z_1) \, dz_1 = 2/3$$
 (2)

where our units are sphere diameters.  $\bar{z}_1$  is the largest of the z coordinates of the three supporting spheres.

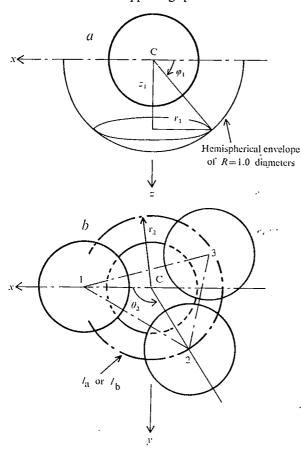


Fig. 2 The coordinate system. C denotes the central sphere; 1, 2, 3, the supporting spheres;  $I_a$  or  $I_b$ , the length used in equation (3).

We can partially test our assumptions and argument so far with reference to the 7934 centre packing. We first define the 'shortest zig-zag path' between the arbitrary spheres S and E (Fig. 3). Around the sphere S and on the side of the sphere E we search sphere centres lying within the hemispherical volume of radius R. We simultaneously obtain the distance  $D_i$  from each sphere centre to the line SE and also the SE-directional

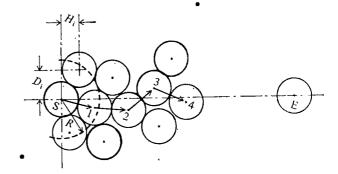


Fig. 3 The shortest zig-zag path.

component  $H_i$  of the distance from the sphere S to each sphere centre. Then we move from the sphere S to the next central sphere 1 which lies nearest to the line SE (that sphere for which  $D_i$  is minimum). The procedure is repeated until we reach the sphere E. In this way we have obtained information on the packing structure along with the length of the shortest zig-zag path between two arbitrary spheres. The first supporting sphere as defined above lies on the shortest zig-zag path, and equation (1) expresses the  $H_i$  distribution of the shortest zig-zag path, which agrees on average with the experimental result fairly well (Fig. 4).

For ease of later calculation, we now fix the first supporting sphere at the point  $(\sqrt{5/3}, 0, 2/3)$ —we place the sphere on the xz-plane. Our final result is independent of this choice provided  $\bar{z}_1 = 2/3$ .

Subject to the existence of the first supporting sphere, we now calculate the expected z coordinate of a second supporting sphere. The projected surface area of the spherical envelope which can be seen from distant z direction becomes (Fig. 2b)

$$S_{2} = \int_{z_{2}=0}^{z_{2}=2/3} l_{a} \sin \varphi_{2} d\varphi_{2} + \int_{z_{2}=2/3}^{z_{2}=z_{*}} l_{b} \sin \varphi_{2} d\varphi_{2}$$

$$= \int_{0}^{2/3} [(l_{a} z_{2} dz_{2})/\sqrt{(1-z_{2}^{2})}] + \int_{2/3}^{z_{*}} [(l_{b} z_{2} dz_{2})/\sqrt{(1-z_{2}^{2})}]$$

$$= \int_{\sqrt{5/3}}^{1} l_{a} dr_{2} + \int_{r^{*}}^{\sqrt{5/3}} l_{b} dr_{2}$$
(3)

where

$$l_{a} = 2 \int_{-r_{2}}^{A} [(r_{2} dx_{2})/\sqrt{(r_{2}^{2} - x_{2}^{2})}]$$

$$= 2r_{2} \left[ \sin^{-1} \frac{(5/9) - (1 - r_{2}^{2})}{2r_{2}\sqrt{5/3}} + \frac{\pi}{2} \right]$$
with  $A = (5/9 - z_{2}^{2})/(2\sqrt{5/3})$ 
and  $l_{b} = 2 \int_{-r_{2}}^{B} (r_{2} dx_{2}/\sqrt{(r_{2}^{2} - x_{2}^{2})})$ 

$$= 2r_{2} \left[ \sin^{-1} \frac{(1 - 4\sqrt{(1 - r_{2}^{2})/3})}{2r_{2}\sqrt{5/3}} + \frac{\pi}{2} \right]$$

with 
$$B = (1-4z_2/3)/(2\sqrt{5}/3)$$
  
and  $r_* = \sqrt{(1-z_*^2)} z_* = (2+\sqrt{15})/6$ 

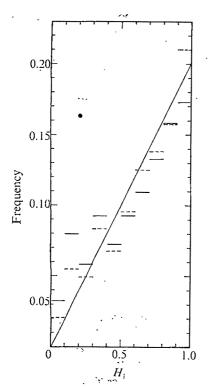


Fig. 4 The  $H_i$  distribution for the spheres on the shortest zig-zag paths. Full line, Equation (1); —— and – – –, experiments for R = 1.005 and R = 1.01, respectively.

The  $I_a$  term is obtained for the restrictions  $(x_2-\sqrt{5/3})^2+y_2^2 \ge 1$  and  $z_2 \le 2/3$ ; the  $I_b$  term for  $(x_2-\sqrt{5/3})^2+y_2^2+(z_2-2/3)^2 \ge 1$  and  $z_2 \ge 2/3$ . Using equation (3), the expected z value of the second supporting sphere becomes

$$\overline{z}_2 = (\int_{\sqrt{5/3}}^1 z_2 \, l_a dr_2 + \int_{r_*}^{\sqrt{5/3}} z_2 \, l_b dr_2) / S_2 = 0.61154365$$
 diameters (4)

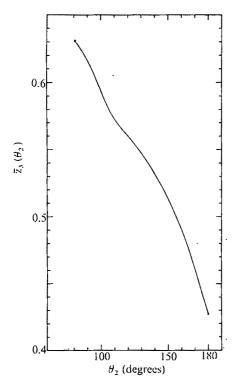


Fig. 5  $\bar{z}_{3}(\theta_{2})/\theta_{2}$  relation.

where  $z_2 = \sqrt{(1-r_2^2)}$ . Although  $\bar{z}_2$  has been obtained, expected values of  $x_2$  and  $y_2$  remain unknown. We now express the coordinates of the second supporting sphere as  $(r_2 \cos \theta_2, r_2 \sin \theta_2, 0.61154365)$ , where  $r_2 = \sqrt{(1-\bar{z}_2^2)}$  (see Fig. 2b).

For a given value of  $\theta_2$  we can now calculate the projected surface area  $S_3$  of the spherical envelope on which the centre of a third supporting sphere lies, subject to the following conditions: (1) the centre of the third supporting sphere lies on the surface of the spherical envelope and we require the projected area which can be seen from distant z direction; (2) the three supporting spheres do not intersect amongst themselves $d_{13} > 1$  and  $d_{23} > 1$  (where  $d_{ij}$  denotes the distance between the two sphere centres, i and j.); and (3) the triangle consisting of the centres of the three supporting spheres, which can be seen from distant z direction, must include the projected centre of the central sphere (Fig. 2b). This is the stability condition for the central sphere. As is seen from Fig. 2b, we need consider only one side of xz plane, and sphere 2 touches sphere 1 st  $\theta_2 = 80.995^{\circ}$ , giving the range of  $80.995^{\circ} \le \theta_2 \le 180^{\circ}$ . On the way to calculating numerically the projected surface area  $S_3$ , we can simultaneously obtain the expected value  $\overline{z}_{3}(\theta_{2})$  of the third supporting sphere for  $\theta = \theta_2$ . In the numerical calculation, minute surface areas satisfying the above three conditions were summed up for a fixed z value. In this way we obtain the  $\theta_2 / \overline{z}_3(\theta_2)$  relation and its mathematical mean value becomes

$$\bar{z}_3 = \int_{80.995^\circ}^{180^\circ} \bar{z}_3 (\theta_2) d\theta_2 / (180^\circ - 80.995^\circ) = 0.54093$$
 (5)

 $\bar{z}_3$  ( $\theta_2$ ) decreases monotonically with increasing  $\theta_2$  (Fig. 5); we find  $\bar{z}_3=0.54093$  corresponds to  $\theta_2=134.53^\circ$ . Thus we have determined the expected coordinates of the second supporting sphere. We now express the expected coordinates of the

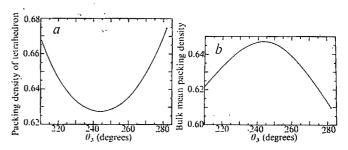


Fig. 6 a, Probable packing density of the tetrahedron. b, Probable density of the random packing.

third supporting sphere as  $(r_3 \cos \theta_3, r_3 \sin \theta_3, 0.54093)$ , where  $r_3 = \sqrt{1-\overline{z}_3^2}$ , and sphere 3 touches sphere 2 and sphere 1 at  $\theta_3 = 210.44^\circ$  and 281.93°, respectively, giving a possible range of  $210.44^\circ \le \theta_3 \le 281.93^\circ$ .

Next we consider the tetrahedron consisting of the centres of the central sphere and the three supporting spheres. The packing density  $\Psi$  of the spheres in the tetrahedron can be expressed by a function of  $\theta_3$ , the  $\Psi/\theta_3$  curve being U-shaped with upper bounds of about 0.667 and 0.675 with a minimum of 0.627 (Fig. 6a). The position of the third supporting sphere, in other words  $\theta_3$ , is still unknown at this stage.

The formulae used in the above calculation are collected in ref. 23.

#### Overall packing density

So far we have discussed only the most probable shape of the tetrahedron, a collection of which constitutes the random packing. As the tetrahedron cannot be considered to be a 'unit cell' of the packing, its density will not necessarily correspond to the bulk density. Instead, we refer to Finney's polyhedral analysis<sup>16</sup> in which the packing of spheres is considered in terms of the packing of the 'Voronoi polyhedron' or

'Dirichlet region' (refs 24, 25), and relate part of the most probable tetrahedron discussed above to the most probable Voronoi polyhedron. We postulate that this average polyhedron is characteristic of the packing, and thus gives us the bulk packing density.

First we bisect perpendicularly the distances between the central sphere and the three supporting spheres by three planes, dividing the tetrahedron into two parts. The inner part, the central sphere side, is considered as a representative angular segment of the Voronoi polyhedron confining the central sphere, since the most probable tetrahedron is discussed for an arbitrary direction and the system must be isotropic overall. Thus we can calculate the packing density of the angular segment, that is the bulk mean density  $\psi_{\text{bulk}}$  of the random packing. As is shown in Fig. 6b, the  $\Psi_{bulk}/\theta_3$  curve is convex with lower bounds of 0.6099 and 0.6220, with a maximum of 0.6472, and a mathematical mean value of 0.6357. Since no criterion is available for determining  $\theta_3$ , we suggest the mathematical mean value is the expected bulk mean density of the random packing; namely, that the random packing density of equal spheres is 0.6357 (with possible bounds of 0.6099 and 0.6472), in excellent agreement with the recognised experimental maximum value of 0.636  $\pm$  0.001 (refs 7-16). In addition, the stability criterion implies upper and lower density bounds of 0.6472 and 0.6099. The latter we suggest might be identified with the random loose packing.

### Validity and application of the technique

Our argument is not rigorous, but its consistency with experiment is encouraging. In particular, we stress the following problems: (a) the average coordination of 6 is still open to argument, although the experimental data support it, even for the close-packed aggregate; (b) we have ignored the restrictive effects of neighbouring configurations, and so probably underestimated cooperative geometrical effects; (c) we have prematurely averaged our distributions. More elaborate calculations could avoid this objection, and would presumably give a  $\theta_3$ :  $\Psi$  distribution having a slightly different shape and bounds.

Thus we must be wary of assigning too much significance to the actual values obtained. The mean value 0.6357 is temptingly close to the accepted experimental maximum density values of Scott12 and of Finney16 (0.6366  $\pm$  0.0008 and 0.6366  $\pm$  0.0004 respectively) but we cannot assert definitely that the mean value should necessarily correspond to that maximum density obtained in the laboratory. We might rather suggest that the laboratory maximum density should correspond to our upper bound of 0.6472, while the lower bound of 0.6099 should refer to the minimum packing density for an isotropic stable packing—the so-called random loose-packing. If this assignment is correct, both bounds are about 0.01 too high, a discrepancy which may be due to our admittedly premature averaging; alternatively, both the Scott and Finney packings may not have been true maxima.

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### Structural uniqueness of lactose operator

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The repressor binding capacity of hplac DNA was maximally reduced by as few as 2-5 cuts by either of two single-strand specific nucleases. But approximately 300 cuts by three nonspecific nicking agents were necessary to give the same effect.

THE properties and conformation of DNA are dictated by nucleotide sequence<sup>1,2</sup>. This conclusion was derived from at least fourteen different chemical, physical, enzymatic and biological studies on twelve high molecular weight duplex DNAs with defined repeating nucleotide sequences. The effect of sequence on structure was apparent with these DNA polymers since the influence was magnified compared with natural DNAs whose properties are a composite of many types of sequence effects.

We are extending these studies by attempting to determine if

natural DNA has the same conformation throughout its entire length or if different structures exist at various sites along the chain. It is presumed that regions with 'anomalous' conformations are present in small amounts compared with the bulk DNA (which may be in a typical DNA B configuration). Thus, a highly sensitive and specific assay is needed to detect regions with unique conformations. Also, if these odd regions are present in small amounts, they may serve as regulatory sites, instead of structural genes. Their uniqueness may contribute to the high degree of specificity and tightness of binding of regulatory proteins.

The lac repressor plus λplac DNA system<sup>3</sup> provides a highly sensitive and selective assay for a specific region of DNA, since the repressor binds to operator DNA with a remarkably large equilibrium constant4 and does not perform complex catalytic or translocating functions. Nucleases with defined specificities were used as selective probes for assessing the

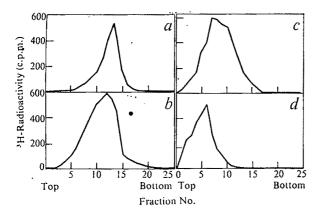


Fig. 1 Alkaline sucrose gradient profiles of <sup>3</sup>H-λplac DNA rig. 1 Aikaine sucrose gradient profiles of 'H-Apiac DNA treated with  $S_1$  nuclease. For preparation of  $\lambda$ plac 5 phage, medium J (ref. 14) was used but with 10 µg ml<sup>-1</sup> of thymidine and 0.4% of glucose instead of lactose. <sup>32</sup>P and <sup>3</sup>H-thymidine-labelled  $\lambda$ plac 5 DNA was prepared from strain JG 108  $\lambda$ plac (provided by A. Riggs). A 100 ml culture was grown at 30° C to an A of 0.4 and induced at 45° C for 15 min. 2 mCi of <sup>32</sup>P-phosphate or <sup>3</sup>H-thymidine was added on induction and the culture was shifted to  $37^{\circ}$  C and incubated for an additional the culture was shifted to 37° C and incubated for an additional 3 h. Cells were centrifuged, resuspended in 10 ml of Φ80 buffer (50 mM NaCl, 1 mM MgSO<sub>4</sub>, 20 mM Tris-HCl, pH 7.4) and lysed with chloroform. After centrifugation, the phage were concentrated with polyethylene glycol 6,000. The precipitated phage were centrifuged and resuspended in 5 ml of Φ80 buffer. Solid CsCl (optical grade) was added to a final density of 1.49 g ml<sup>-1</sup> and this suspension was centrifuged for 14 h at 35,000 r.p.m. in a Spinco Ti-50 rotor. Phage, purified from two cycles of CsCl gradients, were dialysed against DB I (10 mM Tris-HCl, pH 7.4, 10 mM MgSO<sub>4</sub>). The DNA was extracted from the phage by rolling the solution with an equal volume of phenol six times and the excess phenol was removed by six ether extractions. The aqueous layer was dialysed exhaustively against and subsequently stored in DB II (2 M NaCl, 10 mM Tris-HCl, pH 7.4, 0.1 mM EDTA). Immediately before use, DNA stored in DB II was dialysed against DB III (10 mM Tris-HCl, pH 7.4, 0.1 mM EDTA). All dialysis tubing was autoclaved and stored in sterile buffer before use. DNA prepared by this procedure contained few, if any, detectable single-strand scissions (a). S<sub>1</sub> nuclease was purified to 23 units mg<sup>-1</sup> by the procedure of Vogt<sup>6</sup>. Reactions with S<sub>1</sub> nuclease contained 10 mM ammonium acetate (pH 5.0), 1 mM ZnSO<sub>4</sub>, 50 mM NaCl, 15 µg ml<sup>-1</sup> of <sup>3</sup>H-λplac DNA and variable amounts of S<sub>1</sub> nuclease. We define one unit of S<sub>1</sub> nuclease as the amount of enzyme required to render acid soluble 25 nmol of <sup>3</sup>H-(dA-dT)<sub>n</sub> (dA-dT)<sub>n</sub> per min at 37° C in a 100 μl reaction mixture containing 650 nmol of 3H-(dA-dT)n (dA-dT)n instead of \(\lambda plac DNA\). Reactions (0.10 ml) were performed at C for 30 min and were terminated by addition of EDTA (to 25 mM). The reaction products were extracted three times with phenol and six times with ether. Recovery was greater than These samples were subsequently used for molecular weight determinations or repressor binding studies. Linear 5 to 20% (w/v) alkaline sucrose gradients were made in 0.3 M NaOH, 0.7 M NaCl, and 1 mM EDTA. DNA samples (50  $\mu$ l) were layered on to the gradients and were centrifuged at 45,000 r.p.m. for 2.5 h at 4° C in an SW 50.1 rotor and the radioactivity of 0.2 ml fractions was determined. Molecular 0.2 ml fractions was determined. Molecular weights of the DNA fragments were calculated relative to markers of intact λDNA and ΦX174 DNA (from J. Dodgson). a, No S<sub>1</sub> nuclease; b, 0.1 units nuclease; c, 1 unit nuclease; d, 10 units nuclease. Fraction 13,  $15 \times 10^6$ ; fraction 10,  $7 \times 10^6$ ; fraction 7,  $3 \times 10^6$  and fraction 5,  $1 \times 10^6$ .

structural uniqueness of the operator DNA. The comparative ability of two single-strand specific nucleases (mung bean and  $S_1$  nuclease) and of three nonspecific nicking agents (pancreatic DNase, micrococcal nuclease and sonication) to abolish repressor binding ability as a function of reduction in DNA molecular weight was determined.

#### Single-strand specific nucleases

 $S_1$  nuclease cleaves double-stranded  $\lambda plac$  DNA into large fragments even though it has an absolute preference for single-stranded over double-stranded DNA<sup>5,6</sup>.  $^3H$ - $\lambda plac$  DNA was degraded by  $S_1$  nuclease to different extents and the sizes of

the DNA fragments were determined on 5-20% alkaline sucrose gradients (Fig. 1). Under the conditions used, treatment with 1 and 10 units of nuclease caused an average molecular weight reduction from  $30\times10^6$  (undegraded) to  $6\times10^6$  and  $4\times10^6$ , respectively. If the DNA were single stranded, the latter concentration of enzyme was sufficient quantitatively to degrade the DNA to acid-soluble fragments (results not shown).

Gel electrophoretic analyses were performed on 0.6% agarose gels on selected DNA fragments, which had been denatured, to provide a separate determination of molecular weights and to provide greater resolution. Figure 2 shows the distribution of S<sub>1</sub> nuclease-generated fragments and the distribution of molecular weight markers. Substantial agreement was found between the positions of the shoulders on the sucrose gradient analysis (Fig. 1) at fractions 5, 7 and 10 and the sizes of the predominant species on gel analysis (Fig. 2) at fractions 45, 37 and 30, representing molecular weights  $1.2 \times 10^6$ ,  $3 \times 10^6$  and  $7 \times 10^6$  respectively. Also, both analyses showed the virtual absence of undegraded DNA (15×106, single-strand molecular weight) and of small fragments (<100,000) consistent with the expected mode of action of S<sub>1</sub> nuclease<sup>5,6</sup>. These analyses reinforce the validity of the size determinations. Also, the gel analyses suggest that the DNA was cut at specific sites since a nonrandom distribution of S<sub>1</sub> fragments was generated (compare with distribution of Eco R<sub>1</sub> fragments in Fig. 2).

The ability of the  $S_1$  nuclease fragmented  $^3H$ -DNA to compete against undegraded  $^{32}P$ -DNA for *-lac* repressor is shown in Fig. 3. The more highly degraded DNAs bind repressor less well.

Figure 4 correlates the data from the first three figures and shows that a small reduction in molecular weight caused by  $S_1$  nuclease provides a large reduction in repressor binding activity. The binding was reduced 50% when the  $\lambda plac$  DNA (molecular weight,  $30\times10^{\circ}$ ) was degraded into fifths (molecular weight,  $6\times10^{\circ}$ ). If this number of scissions occurred on a random basis, the repressor binding ability of the DNA should be unaltered (<1% reduction).

Studies were performed also with the mung bean nuclease which prefers single-stranded over double-stranded DNA by a

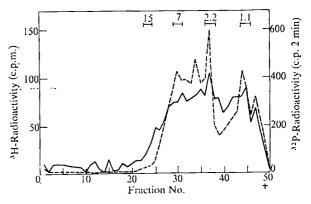


Fig. 2 Agarose gel electrophoresis of  ${}^3\text{H}-\lambda\text{plac}$  DNA treated with  $S_1$  nuclease. (—),  ${}^3\text{H}-\lambda\text{plac}$  DNA treated with 1 unit of  $S_1$  nuclease, as described in Fig. 1c; (---), fragments of  ${}^3\text{P}-\lambda\text{plac}$  DNA treated with the Escherichia coil  $R_1$  restriction enzyme (from B. Weisblum). The Eco $R_1$  reaction mixture contained 100 mM Tris-HCl (pH 7.4), 5 mM MgCl<sub>2</sub>, 5.75 µg ml-1  ${}^3\text{P}-\lambda\text{plac}$  DNA and sufficient enzyme to degrade all  ${}^3\text{P}$ -DNA .The 50 µl reactions were incubated at 37° C for 15–30 min.  ${}^3\text{H}$ -sample DNA and Eco $R_1$  restricted  ${}^3\text{P}-\lambda\text{plac}$  DNA markers were denatured with 0.2 volume of 2 M NaOH and were made 10% in sucrose and 0.04% in bromophenol blue. The mixture was electrophoresed for 5 h at 5 mA per gel at room temperature on 0.6% w/v agarose gels16, with continuous circulation of the electrophoresis buffer (36 mM Tris, 30 mM NaH<sub>2</sub>PO<sub>4</sub>, 1 mM EDTA, 0.05% SDS (pH 7.7)) between the upper and lower chambers. Transverse 3 mm gel slices were dissolved in 1 ml water by autoclaving and radioactivity was determined. The 15 × 106 molecular weight marker was unfragmented, but denatured,  $\lambda\text{plac}$  DNA. Solid line,  ${}^3\text{H}$ ; broken line,  ${}^3\text{P}$ .

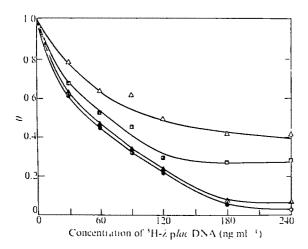


Fig. 3 Repressor binding ability of λplac DNA treated with S<sub>1</sub> nuclease. <sup>3</sup>H-Aplac DNA was treated with variable amounts of S<sub>1</sub> nuclease (see Fig. 1) and the affinity of the nuclease-nicked <sup>3</sup>H-λplac DNA for the lactose I<sup>sQ</sup> repressor (provided by A. Riggs) was determined by competition equilibrium experiments as described. The operator active repressor was determined as described and was at least 95% pure. Various amounts of test  $^3\text{H-}\lambda\text{p}/ac$  DNA were mixed with 0.03 µg of intact  $^3\text{P-}\lambda\text{p}/ac$  DNA in 0.5 ml of BB1. After addition of repressor (1.2  $\times$  10<sup>-15</sup> mol), the mixture was incubated for at least 30 min at room temperature; 0.2 ml aliquots were then filtered in duplicate through nitrocellulose membranes (pretreated as described14 The membrane filters were washed once with 0.4 ml of FB1 dried, and radioactivity was determined. Each experimental point is the average of two filters; reproducibility was  $\pm 10\%$ .  $\blacktriangle$ , No enzyme;  $\bullet$ , 0.1 units;  $\blacksquare$ , 1 unit;  $\uparrow$ , 10 units of  $S_1$  nuclease. The repressor binding ability  $\theta$ , is the ability of the nuclease-treated <sup>3</sup>H-DNAs to compete against intact <sup>35</sup>P-λplac DNA for the lactose repressor.  $\theta$  is obtained by dividing the 32P-counts retained in the presence of competing 3H-DNA by that retained in the absence of competing DNA.

ratio of 30,000 to 1 (ref. 7). The mung bean nuclease is at least as effective as the  $S_1$  nuclease (Fig. 4) in destroying repressor binding. When approximately two double-stranded cuts were made, which decreased the molecular weight by one-third  $(10\times10^6)$ , a 50% reduction of repressor binding was found. Further degradation of the DNA with more enzyme to a molecular weight of  $0.5\times10^6$  gave only a slightly greater (60-70%) reduction in binding. The reason for the lack of total reduction in repressor binding by these two nucleases is unknown. That the mung bean enzyme is slightly more specific than the  $S_1$  nuclease may be due to its higher extent of purification and hence single-strand specificity; trace contamination with a double-strand specific endonuclease could diminish substantially the specificity demonstrated in Fig. 4.

### Nonspecific nicking agents

Since the S<sub>1</sub> and mung bean nucleases had a preferential effect on repressor binding sites, λplac DNA fragmentations were performed with pancreatic DNase, micrococcal nuclease and sonication, agents which do not have pronounced specificities. λplac DNA was degraded with pancreatic DNase over a 10<sup>16</sup> fold concentration range. The size of the products was analysed by alkaline sucrose gradient sedimentation and ranged from approximately 15 × 10<sup>6</sup> to 100,000 (duplex molecular weights) (Fig. 4). Repressor binding activity of these fragments was substantial (greater than 85%) even when the DNA was fragmented to 3% (1 10<sup>6</sup>) of its original size. To observe a 50% reduction of repressor binding ability, λplac DNA had to be degraded into fragments no larger than 100,000 in molecular weight (Fig. 4).

As it was important to determine accurately the size of the sample most highly degraded by pancreatic DNase and since this sample remained near the meniscus on an alkaline sucrose gradient, indicating a molecular weight no larger than 100,000, a determination was performed on 10% polyacrylamide gels

under established conditions. The DNA was at eps (c. 24s) pairs long indicating a molecular weight of at c. s. (d) Hence, the dual analyses set the molecular weight of (c. s. 22s) at 100,000.

Similar studies were carried out with two steal is agents for degrading DNA which gave vittal ۲¢. results. Degradations were performed with nuclease, which generates 3'-phosphoryl terrais ultrasonication, a random mechanical process c micrococcal nuclease (Worthington) contained 50 HCl (pH 7.4), 2 mM CaCl<sub>2</sub>, 15 μg ml<sup>-1</sup> of <sup>3</sup>H ?. 'ε variable amounts of micrococcal nuclease. i., ... degraded into double-stranded fragments of .r.  $15 \times 10^6$ ,  $3 \times 10^6$  and  $0.5 \times 10^6$  by both techniques . 1 shown). Repressor binding studies demonstrated  $15 \times 10^6$  and the  $3 \times 10^6$  molecular weight previous no detectable reduction in repressor binding ac vitra the  $0.5 \times 10^6$  preparations retained at least  $80^{\circ}$ , if the The results of these sonication studies are cors or previous work8 which demonstrated that  $\lambda plac$ sonicated into fragments of one million molecu a. ar. still retain full repressor binding.

Hence, the results from these three non-specific real sections are internally consistent and emphasized of nicking by  $S_1$  and mung bean nucleuse of the section of and 150 times as many cuts by the honspecific real sections binding as for the two single-strand specific enzimes

### Protection of operator by repressor

Since only a few nucleolytic scissions by the SD C and specific nucleases sufficed to diminish represent the specific nucleases are specific nucleases.

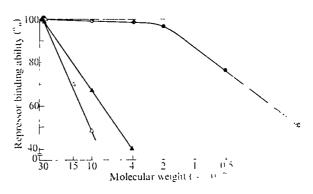


Fig. 4 Comparative ability of S<sub>1</sub> nuclease, murgers and pancreatic DNase to diminish repressor by the state of the processor by the state of the published method? An accurate specific action of the published method? An accurate specific the enzyme could not be determined since the high contained no detectable protein. Reach seem the enzyme contained no detectable protein. Reach seem to make a summary and various amounts (0.1 to 10 units) of mung bear of mung bear nuclease as the amount of enzyme seem of mung bear nuclease as the amount of enzyme seem acid soluble 65 nmol of "H-(dA-dT)<sub>n</sub> (dA-dT) seem acid soluble 65 nmol of "H-(dA-dT)<sub>n</sub> (dA-dT) seem acid soluble 65 nmol of "H-(dA-dT)<sub>n</sub> (dA-dT) seem acid soluble 65 nmol of "H-(tanget) seem action containing 650 nmol of the seem action to make the protein seem action to the protein seem action to the protein seem action to the protein seem actions with pancreatic DNase (Worthington) contained soluble amounts (from 1 × 10-7 pg to 1 ng seem actions with the protein seem actions with the same value of 0.5 by the amount of the nicked "H-INA action the same value of 0.7 Mung bean nuclease; A 5.7 methods alkaline sucrose gradient and 10% polyacrylamide gel stee sphoresis.

Table 1 Repressor binding ability of  $\lambda plac$  DNA treated with  $S_1$  nuclease in absence or presence of repressor

•		Molecular weight of	Repressor
Reaction	Additions ·	DNA after treatment	binding %
1	None	$30 \times 10^{6}$	100
2	S <sub>1</sub> nuclease	$4 \times 10^6$	43
3	S <sub>1</sub> nuclease +		
	repressor	$4 \times 10^6$	> 95
4	S <sub>1</sub> nuclease + denatured repressor		
	denatured repressor	$4 \times 10^6$	43

Reaction conditions were identical to those described in Fig. 1 except that no NaCl was present and 10 units of  $S_1$  nuclease was used in all cases. 0.010 ml of repressor buffer (0.13 M potassium phosphate (pH 7.2) and 5% glucose) was added as the blank to reaction 2. Lactose repressor (threefold binding saturation excess at pH 7.0) was added to the DNA and incubated at 0° C for 15 min before the nuclease was added. Denatured repressor was obtained by heating the active repressor at 100° C for 10 min. Molecular weights and repressor binding capacities were measured as described in Figs 1 and 3.

studies were performed in the presence and absence of repressor (Table 1). With a constant amount of S<sub>1</sub> nuclease, the DNA was degraded to a similar extent (molecular weight,  $4 \times 10^6$ ) in the absence or presence of repressor. This result was not surprising since the repressor should protect only a small portion of DNA (about 27 base pairs9). But when the ability of the fragmented DNAs to bind repressor was compared, substantial differences were found between fragments that were generated in the presence or absence of active repressor. A 60% reduction in repressor binding was observed when the DNA was degraded in the absence of added repressor. In contrast, when the nuclease degradation was performed in the presence of active repressor, more than 95% of repressor binding ability was retained. Also, heat-denatured repressor did not protect since the same amount of reduction in repressor binding was found when S<sub>1</sub> nuclease degradation was performed in the presence of heat-denatured repressor as in its absence. Similar experiments with the mung bean nuclease gave a result in complete agreement with the data of Table 1.

The significance of this protection is twofold. Reduction

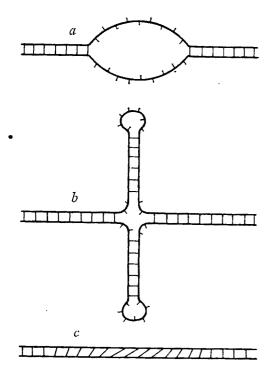


Fig. 5 Possible unique structures. a, Thermolability; b, cruciform; c, helical, non-DNA B.

of repressor binding by single-stranded nuclease treatment must be due to nucleolytic scission at sites identical or closely adjacent to the repressor binding site. Second, the recognition of the operator by the single-strand specific nucleases is not due to some artefactual conformation induced by the nuclease reaction conditions since the repressor binds specifically under the nuclease reaction conditions.

#### Effect of salt

The 'structural uniqueness' of the *lac* operator could be due to (a) a high degree of thermolability (facile breathing), (b) a unique but nonlinear structure containing some unpaired nucleotides such as a Gierer loop¹0 (cruciform) or (c) another unique conformation which is linear and fully base paired but has a different helical pitch or tilt of base pairs, such as found for certain DNA polymers¹¹². High salt concentrations should diminish the local breathing of thermolabile regions of DNA (theory a) and should diminish the tendency to form cruciforms (theory b) (in the absence of cruciform stabilising factors such as modified bases or proteins), but should have little or no effect on the tendency to adopt other unique but linear structures.

Comparative studies were performed with the  $S_1$  nuclease in 50 and in 250 mM NaCl (results not shown). Since the  $S_1$  nuclease was half as active in 250 mM as in 50 mM NaCl on a single-stranded substrate, three times as much nuclease was added to the high salt reactions to assure that the salt effect was solely on DNA conformation rather than the enzyme activity. Under both conditions, the  $\lambda plac$  DNA was fragmented to similar extents and the repressor binding ability of the treated DNA was similarly abolished; 70% reduction of repressor binding was found when the DNA was degraded in 250 mM NaCl to  $1.4 \times 10^6$  (compare with Fig. 4).

### **Models for DNA Structure**

The original goal of this study was to determine if different DNA conformations exist along a chromosome chain. Our results demonstrate that certain regions of  $\lambda plac$  DNA are quite sensitive to single-strand specific nucleases and, therefore, have structures that are different from the greater part of the DNA. This conclusion agrees with and was predicted from the results on DNA polymers<sup>1,2</sup> which revealed the influence of nucleotide sequence on DNA properties and structure.

The nature of the structural uniqueness which is recognised by the  $S_1$  and mung bean nucleases is unknown at present. Three possible models (Fig. 5) are envisaged: model a, the unique region possesses low thermostability and, hence, is in a nonhelical state for a larger proportion of the time than other regions of DNA. The low thermostability could be due to low G-C content or to the presence of modified nucleotides. Model b, the unique region could exist, at least transiently, in a cruciform structure<sup>10</sup> or other configuration which contains some nonpaired bases. Model c, the unique region may be totally helical but in a different geometric configuration (not DNA B) than the great part of the DNA chromosome. For example, the tilt of the base pairs or the helical dimensions could be somewhat altered; such helical variances have been recognised1,2 with the DNA polymers. Whatever this odd configuration may be, it must be susceptible to the single-strand specific nucleases.

The nucleotide sequence of transcripts of the *lac* promoter and operator region has been determined (ref. 9 and Dickson, Abelson, Barnes, and Reznikoff, personal communication). Though revealing several interesting sequence anomalies, these studies do not provide direct information on the conformation of these regulatory sites. Two facets of the sequence may however be relevant to this work; the potential for the formation of a cruciform exists at the presumed repressor binding site. In a run of 11 possible base pairs, 10 are true Watson-Crick pairs. Additionally, several regions of high A-T content exist

in the promoter-operator sequence. The longest contains 10 A-T pairs in a run of 12 base pairs. The significance of these regions remains to be determined.

It is not possible to distinguish between these three models on the basis of present information. The nucleolytic sensitivity of the repressor binding activity of \( \lambda plac \) DNA was insensitive to salt concentration (see above) which strongly favours model c and disfavours a and b since the parent linear duplex structures should be stabilised by salt. Also, there is little effect of temperature on the binding of repressor to λplac DNA4 which favours model c but disfavours a and b. Again, the large forward rate constant of the repressor-operator interaction4 makes probable the interaction of the repressor with a stable. not transient, unique operator configuration; this consideration favours model c and disfavours a and b. Model a is favoured by the presence of A-T-rich regions in the sequence and this model easily explains the results described here. The lac repressoroperator interactionis, however, quite sensitive to ionic strength4 which favours this model, but the sensitivity may be explicable on the basis of protein-protein, rather than protein-nucleic acid, interactions. Also, previous studies11 with DNAs of defined sequence demonstrated the need for both high A-T content and certain sequence features for effective repressor binding; low thermostability alone was not sufficient.

The cruciform model (b) is plausible because of its simplicity and because of the sequence studies but is argued against by (1) its thermodynamic improbability and (2) recent studies<sup>12</sup> which demonstrate that λplac DNA containing a high density of negative supercoil twists binds repressor only slightly better than non-supercoiled DNA. Negative supercoils should stabilise cruciform structures.

The extent of 'single strandedness' which is necessary to provide the results described here is unknown also but may be only a few nucleotides. Our results should not be interpreted as necessitating long stretches of random coil structure.

We anticipate that the nucleolytic scissions by the singlestrand specific nucleases are at the repressor binding site, as implicated by the repressor protection experiment (Table 1). But it may be that a neighbouring region is the site of attack and that the influence is transferred along the DNA chain. Experimental justification for the transmission of conformational stability (telestability) has recently been found (Burd, Wartell, and R.D.W unpublished) with the duplex block polymer  $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$ .

That the lac operator may possess elements of single strandedness is consistent with the high degree of specificity of binding of the repressor since nonhelical DNA could provide substantially more characteristic sites for binding than helical DNA13.

This work was supported by the National Science Foundation. We thank Professor J. Dahlberg for determining the size of a DNA sample on polyacrylamide gels.

Note added in proof: Preliminary results show that the halflife for the S<sub>1</sub> nuclease nicked DNA-repressor complex is reduced from 20 min to approximately 10 min, thus explaining the lack of total abolition of repressor binding.

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## letters to nature

### QSOs of high redshift?

We have completed a series of observations at Jodrell Bank on the radio structures of a complete sample of OSO candidates identified from the Parkes ± 4° equatorial survey. Four sources are of particular interest in that they have properties which lead us to suggest that they may be QSOs of unusually high redshift.

At present we are not aware of any QSOs with steep radio spectra which have redshifts greater than 2.4; all those with higher redshifts have flat or concave radio spectra. (We use the spectral categories defined by Stannard<sup>1</sup>, except that the spectral index is determined at 1 GHz observed frequency

rather than 3 GHz emitted frequency.) It is of interest to consider the properties that QSOs with steep radio spectra and high redshifts may possess. In common with most of the known high redshift QSOs they would probably lack an ultraviolet excess2. A further property may be deduced from studies of angular diameter3,4 which suggest that, at high redshift, steep spectrum QSOs are likely to be compact, with angular diameters of less than a few arcs. Most of the flat and concave spectrum QSOs of high redshift have visual magnitudes between 18 mag and 19 mag. The studies of Setti and Woltjer<sup>5</sup> and Stannard<sup>1</sup> suggest, however, that any steep spectrum QSOs of comparable redshift will be, on average, about one magnitude fainter. One reason for the apparent lack of steep

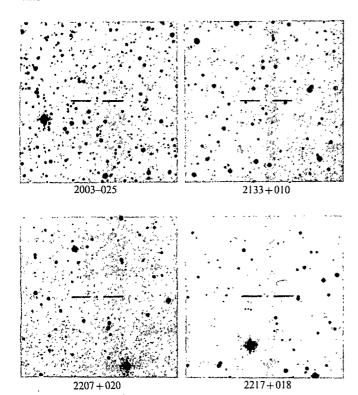


Fig. 1 Finding charts (approximate scale 10 arc s mm<sup>-1</sup>) reproduced from the red prints of the Palomar Sky Survey.

spectrum QSOs of high redshift may be that few QSO candidates fainter than 19.5 mag have been studied spectroscopically.

The Parkes 2,700 MHz survey of the  $\pm$  4° equatorial strip contains 341 sources above the completeness level of 0.35 Jy(1 Jy =  $10^{-28}$  W Hz<sup>-1</sup>m<sup>-2</sup>). We have used accurate radio positions measured at the Royal Radar Establishment, Malvern, to check the original Parkes identifications, and in some cases to propose new or revised identifications. One hundred of the sources can be identified confidently with stellar objects down to the limit of the Palomar Sky Survey prints. The radio structures of these QSO candidates have been studied at 962 and 1,666 MHz with the Mark II-III radio link interferometer at Jodrell Bank. Full details of these observations will be published elsewhere. Four steep spectrum sources were found to be essentially unresolved, and all have optical properties, which, from the discussion above, would be expected of high redshift QSOs.

The positions of the four QSO candidates are given in Table 1, and finding charts are given in Fig. 1. The properties of the objects are summarised in Table 2. They all have spectral indices equal to or steeper than -0.7, and none shows evidence of a low frequency cutoff. This suggests that the sources do not possess intrinsically compact structures. Three are completely inresolved in our interferometric observations and thus have argest angular sizes of less than 0.5 arc s. The fourth (2207 + 120) may possess extended structure in addition to a compact component giving rise to 70% of the total intensity. Visual

Table 1	Radio positions of compact QSO candidates						
Source	Radio posit	Position accuracy					
	RA (h min s)	dec. (0° ′ ′′)	(r.m.s.)				
2003 025		-02 32 15.2	(arc s) 0.8				
2133 + 010	21 33 19.56	01 04 48.1	1.3				
2207 + 020	22 07 00.20	02 03 55.9	1.5				
2217 + 018	22 17 58.05	01 49 46.6	1.4				

magnitudes for the sources have been estimated from the Palomar Sky Survey prints. All are 19.5 mag or fainter. Colour estimates have been provided by J. G. Bolton for almost all the QSO candidates in our sample. Three of the four sources discussed here are amongst the small number (only seven) found to have no ultraviolet excess. Another of the seven, which has a flat radio spectrum, has a redshift of 2.66 (ref. 7).

Table 2 Radio and optical properties of compact QSO candidates

Source	Radio spectral	Largest angular	Visual	Ultraviolet
	index		magnitude	excess
2003 - 025	0.7	< 0.5	19.5	No
2133 + 010	-0.7	< 0.5	20.0	Yes?
2207 + 020		$(\sim 1)$	19.5	No
2217 + 018	-0.7	≥0.5	19.6	No?

Individually, the properties of small angular size or optical faintness cannot be taken as strong evidence that a QSO has an unusually high redshift. When, however, these properties are combined with a steep radio spectrum and lack of ultraviolet excess, we consider that such an object may well have a high redshift. Optical spectroscopy on 2003-025 and 2207+020 has recently been reported by Strittmatter et al.8. They confirm that both objects have ultraviolet deficits, but are unable to assign redshifts. Despite this, we suggest that all four objects are worthy of further spectroscopic investigation.

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### Change of pulse characteristics of the Vela pulsar with frequency

Below 2,000 MHz the average pulse profile from the Vela pulsar (PSR 0833-45) shows a single peak with a sharp rise and a more gradual decay<sup>1</sup>. At higher frequencies a broader and more complex pulse is observed, evidently comprising two distinct components<sup>2,3</sup>. Observations at 1,400 and 4,830 MHz made with the Parkes 64-m telescope in November 1972 to clarify this frequency dependence can also be related to some  $\gamma$ -ray observations made by Albats *et al.*<sup>4</sup> at the same time.

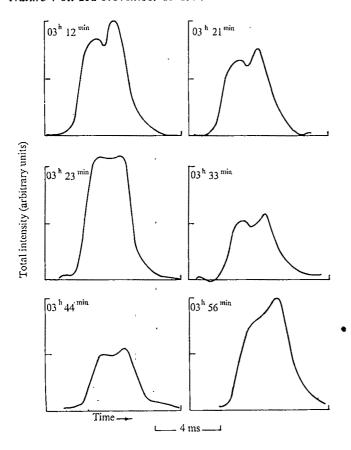


Fig. 1 Average 'total intensity' profiles measured at 4,830 MHz. Each profile is the sum of two integrations at orthogonal position angles of the feed. At each position angle 500 pulses were superposed. The times are Eastern Australian Standard Time on November 8, 1972.

Details of the radio observations are given in Table 1. A single receiver and linearly polarised feed were used at each frequency.

Figure 1 shows some examples of the 'total intensity' pulses measured at 4,830 MHz. The double structure is apparent, as is the time variability (over periods of a few minutes). Some of the variability must be intrinsic to the pulsar, since the relative amplitude of the two peaks also varies. Before the polarisation analysis was carried out the total intensity profiles were normalised.

Ideally, to determine which 4,830 MHz peak most nearly coincides with the 1,400 MHz pulse, simultaneous observations should have been made. But since the two receivers cannot be operated simultaneously the observations were made consecutively and connected by accurate timing measurements.

Table 1 1972 November observations of PSR0833-45*							
Date	Frequency (MHz)	Bandwidth (MHz)	System temperature (K)				
November 8, 1972	4,830	15	90				
November 15–19, 1972 (inclusive)	1,400	0.1	120				

\* Average pulse profiles were measured by superposing many pulses, using a time base operating at the Doppler-shifted pulsar frequency and a sampling rate of 1/400 period (0.223 ms). Such pulse profiles were formed at a number of different feed position angles and the linear polarisation parameters of the average pulse were computed. The measurements were made in pairs, one feed position angle being followed by the position angle orthogonal to it. The sums of such pairs of integrations yield the total intensity pulse.

Several times on each observing day the pulse arrival time was measured against a timing pulse from a caesium-beam clock. Each measurement was accurate to about one-half of a sampling interval, or about 0.1 ms. From the measured arrival times at 1,400 and 4,830 MHz, and from the pulsar's known position<sup>5</sup> and dispersion measure<sup>6</sup>, the barycentric period  $P_0$  and its time derivative  $\dot{P}$  were estimated. For JD 2441638.5 these were  $P_0=0.08922348324\pm7$  s and  $\dot{P}=1.2508\pm0.002\times10^{-13}$ . This value of  $P_0$ , together with the value<sup>7</sup> on JD 2441199.2 and one measured at Parkes on JD 2441934.5 (made available by V. Radhakrishnan and J. Brooks) yields values of  $\dot{P}$  averaged over several months before and after our observations. These are  $1.2477\times10^{-13}$  and  $1.245\times10^{-13}$ , both very slightly smaller than our estimate.

The 'arrival times' used in the estimates relate to the halfintensity points on the leading edge of the pulse. To test the

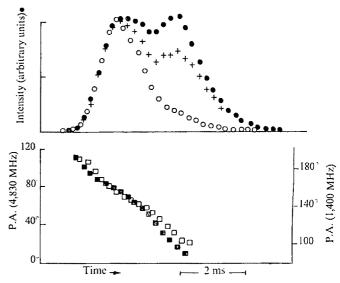


Fig. 2 Average pulse characteristics at 1,400 MHz (open symbols) and at 4,830 MHz (filled symbols). The crosses represent linearly polarised intensity at 4,830 MHz.

possibility that the second 4,830 MHz component more nearly coincides with the 1,400 MHz pulse, we added 1.8 ms—corresponding to the delay of the second component relative to the first—to the 4,830 MHz arrival time and repeated the calculation. There was no significant change in  $P_0$ , but P increased to  $1.256 \times 10^{-13}$ , thus widening the disagreement with the long-term averages. We therefore associate the first component at 4,830 MHz with the single 1,400 MHz pulse.

Figure 2 shows the average profile at 4,830 MHz with that at 1,400 MHz superimposed. The leading edges have been accurately aligned. The shapes of the leading edges and the form of the polarisation position angle 'sweep' at the two frequencies agree well. The position angle at the first 4,830 MHz peak also agrees to within a few degrees with the value extrapolated from lower frequencies. Thus the 4,830 MHz pulse may be represented as the sum of two components (Fig. 3), the first being similar to the 1,400 MHz pulse. The energies  $E_1$  and  $E_2$  of the two components are each equal to about  $20 \times 10^{-30}$  J m<sup>-2</sup> Hz<sup>-1</sup>.

Pulse variability makes it extremely difficult to estimate a meaningful absolute spectral index for frequencies above 4,830 MHz. Writing  $E_1 \propto f^{-\alpha}$  we find values of 2.2 or 4.8 for  $\alpha$ , depending on which measurements<sup>3,5</sup> near 8,000 MHz we combine with the present data. The relative spectral index is more easily estimated. The present results, together with those of Downs *et al.*<sup>3</sup>, indicate that  $E_2/E_1$  varies approximately at

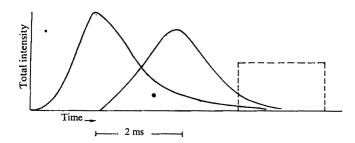


Fig. 3 The two pulse components at 4,830 MHz. The rectangle represents the arrival time of a possible γ-ray pulse as reported by Albats et al.4 The delay of the radio pulse due to dispersion has been removed.

 $f^{2.3}$ . The very much flatter spectrum of the second component suggests that a different emission mechanism is involved, but it is of interest that the position angle sweep through the whole pulse shows little frequency dependence.

Any pulses at frequencies beyond the radio domain might be expected to occur at the time of the second component. On November 16, 1972, Albats et al.4 attempted to detect pulsed  $\gamma$  rays in the 10-30 MeV range from the Vela pulsar. Figure 3 shows the temporal relation between the radio pulse and a possible  $\gamma$ -ray pulse that they report. It seems to lag behind both components of the radio pulse. The estimated uncertainty in our absolute timing measurement does not exceed 1 ms, and Albats et al. quote a similar uncertainty for their data. The time difference exceeds the sum of these uncertainties, but it is of interest that the  $\gamma$ -ray pulse more nearly coincides with the 'flatter spectrum' radio component.

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### New extragalactic variable sources from an 8-GHz survey

Studies of variable extragalactic radio sources have concentrated on sources with 'centimetre-excess' spectra, so that few sources without such spectra have been extensively monitored. We have used the NRAO 300-foot transit telescope at 2.7 GHz to make repeated, accurate observations of 48 sources selected by the Michigan 8-GHz survey without further bias towards any radio spectral type. Variability has been detected in two radio galaxies without centimetre excesses and every quasistellar source in the survey has now been observed to vary at some frequency.

We observed every 8-GHz survey source less than 1' in diameter that would not be significantly confused using the 4.7' resolution of the 300-foot telescope at 2.7 GHz. A threefeed system was used to determine declination pointing corrections at every source transit, so that flux densities could be measured with standard errors of  $\sim 0.03$  Jy from a single transit. The normalisation of the flux density scale among the seven observing periods between September 1972 and June 1974 was established to within 0.5% by observing ~200 nonvariable sources. Details of the observing and calibration procedures will be given by Kesteven and Bridle (in preparation).

Flux densities derived from different transits of a source within each of the seven observing periods have been averaged, as the flux density scatter between transits separated by only a few days is generally consistent with the errors computed for single transits. A  $\chi^2$  test was used to assess whether the scatter of the flux densities from different observing sessions is larger than expected from the errors in the individual measurements. We consider a source to be variable if the  $\chi^2$  probability that the observed scatter arises by chance is less than 0.1%, and possibly variable if it is between 0.1 and 1%. If some sessional flux densities were derived from only one transit the test was repeated deleting each such single observation in turn, and our assessment of source variability is based on the most mutually consistent data set. This procedure ensures that no source is considered variable on the evidence of a single discrepant transit which might have been affected by unknown instrumental effects.

The results for the 48 sources observed are summarised in Table 1. The flux densities are adjusted to the scale of Kellermann et al.3; the errors given for nonvariable sources do not include the uncertainty in normalising to this flux density scale as this does not affect our assessment of variability between the seven observing periods.

Twenty-one of the sources are variable at the 99.9% confidence level and three more at the 99.0% level; seventeen of these 24 are previously known variable sources. The measured flux densities of the five new variable and two new possibly variable sources are given in Table 2. Eleven previously-known variable sources did not vary significantly; the variable component(s) of these sources may make only a small contribution to the total flux density at 2.7 GHz or they may have been quiescent throughout the observing period (see Fig. 3 of Medd et al.4 for examples of such quiescence in variable sources).

The observations confirm the finding<sup>1,2</sup> that the stellar objects in the 8-GHz survey tend to be variable on time scales of a few years whereas the radio galaxies do not. Nine of the fifteen confirmed QSS are variable at 2.7 GHz with variability indices from 0.030 to 0.243, and the remaining six have all varied at one or more other frequencies (see Table 1). Thus all of the confirmed QSS in the 8-GHz survey are now known to be variable. We have also found 8 of the 15 stellar objects without measured red shifts to be variable at 2.7 GHz

			Table 1 Sour	rces observed	in this study (2.7 GHz)		
Source name <sup>1</sup>	Other name	Optical identification <sup>1</sup>	No. of observations	Variable at 2.7 GHz?	Flux density range (Jy)*	V <sub>2.7</sub> ‡	Other refs for variability
0034-01 0035-02 0056-00 0106+01 0111+02	3C15 3C17 PK005600 PK0106+01 OC019	GAL GAL QSS QSS GAL	7 7 7 7 6	Yes No No Yes No	$2.34 / 2.63\dagger$ $3.94\pm0.04$ $1.93\pm0.03$ $2.54 / 3.75$ $0.62\pm0.02$	0.058 <0.021 <0.023 0.192 <0.058	1 1, 2, 4, 8
0112-01 0119+04 0122-00 0237-02 0240-00	P0112-017 OC033 PK0122-00 PO237-027 3C71	BSO BSO QSS BSO GAL	6 5 7 7 7	No Yes Possibly No Yes	$\begin{array}{c} 1.11 \pm 0.04 \\ 1.65 / 1.91 \dagger \\ 1.04 / 1.21 \\ 0.35 \pm 0.05 \\ 2.80 / 3.23 \dagger \end{array}$	<0.058 0.073 0.076 <0.200 0.071	1,2
0305+03 0336-01 0420-01 0422+00 0440-00	3C78 CTA26 PK0420-01 PK0422+00 NRAO190	GAL QSS QSS ? BSO	7 · 7 7 7 7	No Yes No Yes Yes	$4.82\pm0.02$ $1.98 / 3.25$ $1.23\pm0.04$ $0.49 / 0.92$ $2.47 / 2.92$	<0.016 0.243 <0.058 0.305 0.083	1, 2, 3, 4, 5, 6, 8 1, 2, 4 1,2 1, 2, 3, 4, 8
0500+01 0723-00 0736+01 0743-00 0829+04	OG003 DW0723-00 PK0736+01 OI-072	NSO NSO NSO NSO	7 7 6 7 7	Possibly Yes No No No	$ \begin{array}{c} \bullet & 2.14 \ / \ 2.35 \dagger \\ 1.94 \ / \ 2.25 \\ 1.97 \pm 0.12 \\ 1.37 \pm 0.04 \\ 0.61 \pm 0.05 \end{array} $	0.047 0.074 <0.080 <0.051 <0.102	1, 2, 4, 7 1, 4, 5 1, 4
0906+01 0922+00 1055+01 1148-00 1219+04	PK0906+01 OK037 PK1055+01 PK1148-00 PK1219+04	QSS QSS BSO QSS BSO	7 7 7 7 6	No Yes Yes Possibly Yes	0.86±0.03 0.70 / 0.82 2.75 / 3.07 2.35 / 2.56† 0.59 / 0.79	<0.059 0.079 0.055 -0.043 0.145	1, 2, 7 1 1, 2, 3, 4, 5, 6 1, 2
1226+02 1330+02 1434+03 1456+04 1532+01	3G273 3C287.1 PK1434+03 4C4.49 PK1532+01	QSS GAL : BSO?	6 7 7 6 7	Yes No No No Yes	$39.2 / 44.6$ $1.70 \pm 0.03$ $1.83 \pm 0.01$ $0.74 \pm 0.03$ $1.07 / 1.18$	0.064 <0.029 <0.014 <0.060 0.049	1, 2, 3, 4, 5, 6
1535+00 1546+02 1555+00 1635-03 1648+01	OR078 DW1555+00	? QSS BSO GAL	7 7 7 6 7	Yes Yes No No Yes	$0.84 / 1.06 \uparrow$ $1.04 / 1.26$ $1.36 \pm 0.03$ $0.48 \pm 0.02$ $0.83 / 0.96$	0.116 0.096 < 0.030 < 0.083 0.073	1, 2, 7 1, 2, 4, 5
1741 - 03 1949 + 02 2044 - 02 2059 + 03 2128 + 04	P1741 – 038 3C403 3C422 OW098 PK2127+04	OBSC GAL GAL? QSS	7 7 7 7 7	Yes No No Yes No	$\begin{array}{c} 1.90 \ / \ 2.65 \\ 3.35 \pm 0.03 \\ 1.43 \pm 0.04 \\ 0.61 \ / \ 0.72 \\ 2.94 \pm 0.02 \end{array}$	0.165 <0.021 <0.042 0.083 <0.012	1,5
2131-02 2134+00 2210+01 2216-03 2254+02	OX-053 P2134+004 PK2210+01 PK2216-03 OY091.3	BSO? QSS QSS QSS	7 7 7 7 6	Yes Yes No No No	$\begin{array}{c} 1.58 \ / \ 2.39 \\ 7.03 \ / \ 7.47 \\ 1.67 \pm 0.01 \\ 1.23 \pm 0.02 \\ 0.35 \pm 0.01 \end{array}$	0.204 0.030 < 0.015 < 0.025 < 0.056	1, 2 4 1, 2, 4 1
2318+04 2332-01 2335-02	OZ031 OZ-055 OZ-060	BSO BSO BSO	6 7 7	Yes No No	1.07 / 1.32† 0.54±0.03 0.64±0.04	0.105 < 0.101 < 0.071	1

with variability indices from 0.049 to 0.204, a range very similar to that exhibited by the confirmed QSS. Five further stellar objects in the survey are known to have varied at some other frequency. The only 8-GHz stellar objects not yet observed to vary are 0237-02 and 2335-02.

These results are consistent with those of other studies of radio-source variability (such as ref. 9) as all of the stellar objects in the 8-GHz survey have centimetre-excess spectra. The very high incidence of variability among stellar objects with such spectra (93%) suggests that nonvariable objects of this type are rare, if indeed they exist at all.

Our results for radio galaxies are more surprising. Of the ten radio galaxies observed, two (0111+02 and 1635-03) have centimetre-excess spectra and neither has yet been found to vary at any frequency. Unexpectedly in view of most earlier studies, two of the remaining eigh radio galaxies have shown significant variations at 2.7 GHz. These are 0034-01 (3C15) and 0240-00 (3C71). Hazard et al.10 found 0034-01 at 410 MHz to have three main components each ~2" in diameter, the outer components being ~10" apart. The innermost component coincides with the elliptical galaxy that is the identification. 0240-00 has 25% of its 2.7-GHz flux density in a component <1.5" in diameter11 located in the nucleus of the Seyfert galaxy NGC 1068. It has previously been suspected of being variable at millimetre wavelengths<sup>12</sup>. In both 0034-01 and 0240-00 at least part of the radio source lies within the associated galaxy, but the variable components do not dominate the

<sup>\*</sup>The mean flux density is given for sources that did not vary significantly at the 99% confidence level.  $\circ$  †See Table 2 for detailed flux density tabulation. †The 'variability index',  $V_{2,7} = (S_{\text{max}} - S_{\text{min}}) / (S_{\text{max}} + S_{\text{min}})$ , is a normalised measure of the amplitude of the observed variations.

	Ta	ble 2 Flu	x den	sities of new var	iable sources at	2.7 GH:	z		``	
. Source	Date	]	lux de	ensity (Jy)	]	Date	]	lux der	sity (Jy)	
		ú	Vari:	ability significan	t at 99.9% level	•				
0034-01	April	73 2 73 2		0.04 0.10 0.04 0.02			73 74 74	2.34 ± 2.63 ± 2.42 ±	: 0.04	
0119+04	September August November	73	.79 ± .91 ± .78 ±	0.02	Feb Jun		74 74	1.79 ± 1.65 ±	0.02	
024000	April	73 2 73 2	1.08 ± 2.85 ± 2.80 ± 4.08 ±	0.04 0.04			73 74 74	3.23 ± 3.16 ± 3.04 ±	0.03	
1535+00	April	73 (	).93 ±	0.02 0.01 0.02 0.01			73 74 74	0.97 ± 0.92 ± 0.91 ±	0.02	
2318+04		73	.28 ±28 ±32 ±				73 74 74	1.20 ± 1.11 ± 1.07 ±	0.03	
A.C		i	Vari	ability significar	t at 99.0% leve	1			i	<b>.</b>
0500+01	April	73 2 73 2	2.35 ± 2.28 ± 2.14 ± 2.23 ±	0.03 0.07 0.03 0.02			73 74 74	2.33 ± 2.32 ± 2.34 ±	0.03 0.03 0.02	
1148-00	April	73	2.53 ± 2.36 ± 2.51 ± 2:47 ±	0.06 0.04 0.05 0.03			73 74 74	2.56 ± 2.54 ± 2.35 ±	0.03	

high frequency spectrum as in the well known variable radio galaxies such as 3C84 (NGC1275). The variations observed in 0034-01 and 0240-00 are of correspondingly small amplitude, with variability indices of 0.058 and 0.071 respectively. It will be important to determine whether there are variable components in other multi-component radio galaxies like 0034-01, and to establish the location of such variable components within the radio structures.

Five of the six unidentified sources with centimetre excesses are also variable at 2.7 GHz, with variability indices that range from 0.047 to 0.305. The variability of these sources suggests that they are likely to be quasistellar objects.

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### Infrared emission in R CrB type stars

Infrared emission has been observed in R CrB type stars and is considered to be the reradiation of the absorbed visual light by graphite grains1,2.

The lack of correlation between change of star brightness and infrared emission3,4 has been explained in terms of condensation of grains in discrete clouds escaping from the star in random directions. Under this condition we could observe infrared emission without any corresponding change in star brightness. In April and May 1972, however, the observed brightness of one R CrB star decreased about 6 mag, but no changes of infrared flux were observed.

We consider here a possible interpretation of this effect. It seems possible that in the escaped shell nuclei of grains might form in the shape of Platt particles. Such graphite particles with demensions from 3 to 30 Å will be like nonsaturated molecules, the more so as it is likely that a graphite structure with vacancies will grow up. In these

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particles absorption is a quantum-mechanical process resulting from electron jumps between different Fermi levels. For quasi-metallic particles the longest wavelength will be absorbed by one electron, when it jumps from the highest filled to the lowest empty Fermi level. Its wavelength is given approximately by  $\lambda \approx 400L$ , where L is the dimension of the particles. Platt particles will re-emit photons isotropically which were absorbed from an incident beam with very small shifting to the red. These particles cause the extinction a few hundred times greater than classical grains with the same mass; however, they will not reradiate in the infrared region.

The growth of Platt particles to a size of order 10<sup>-7</sup> cm (by accretion C,  $C_2$  and so on, and perhaps coalescence) leads to the formation of the classical nuclei of grains. Further growth will form graphite grains with the radii between  $2 \times 10^{-6}$  and  $2 \times 10^{-5}$  cm whose re-emission in the infrared completely fits the observational data for R CrB and SY Sgr stars<sup>6</sup>.

It is possible that infrared emission is shifted in time relative to the light minimum by an amount appropriate to the necessary period of formation of the classical graphite grains. This time shift depends on the initial conditions of the escaped shell, particularly on the density • and velocity of escape. For that reason this time shift may be different for particular light minima.

This interpretation is confirmed by the fact that light polarisation of R CrB star observed by Coyne and Shawl<sup>7</sup> is strictly correlated with the light curve. At light minimum, and some time after, its light polarisation increases with the decrease of the wavelength which agrees with the behaviour expected for polarisation caused by Platt particles8. For the period before minimum, polarisation is considerably lower and its dependence on the wavelength indicates an origin from grains with relatively large sizes.

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### Polarisation in the night sky at 5,300 A

We have presented observations1 of the linearly polarised intensity, pI, at 5,080 Å (that is, polarisation,  $p \times$  intensity, I), of radiation in the night sky. Contour plots of pI showed a displacement of the minimum of pI away from the antisolar point (ASP). A significant angular structure, and day-to-day variations were also evident. Here, we report observations of pI and of the orientation angle,  $\chi$ , of the polarisation plane of radiation at 5,300 Å in the night sky. Measurements have been taken since May 1973 with a photoelectric polarimeter at the University of Hawaii (J. L. Weinberg, unpublished). Several modifications have been made recently, the most important being the analog-digital conversion of data recording. Digitisation allows faster data reduction and improves measurement precision, which is now limited by instrumental polarisation and photon statistics.

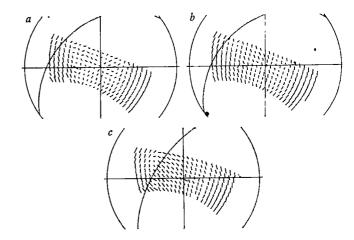


Fig. 1 Linear polarisation of the night sky at 5,300 Å. The diagrams are centred at the ASP. The north ecliptic pole is at the Vertical line, antisolar meridian; horizontal line, the ecliptic plane; dotted line, galactic equator. Part of the circle representing 90° solar elongation is shown. Each measurement is represented by a line with a length proportional to  $\log{(1+pI)}$ , where pI is polarised intensity in  $S_{10}$  (V) units. a, August 25, 1973; b, August 26, 1973; c, July 29, 1973.

Full data will be published elsewhere; here, we discuss 63 night of observation from May 1973-June 1974.

Our observations consist of regular measurements of th Stokes parameters, I, Q, and U, of the night sky radiation a 5,300 Å (half-intensity filter width 53 Å) at fixed altitudes or the N-S meridian. Meridian observations are preferable because they allow measurement of each region at its maximum altitude when the influence of tropospheric scattering is minimal Initially, we observed only on the 180° meridian at altitudes o 35°-75° at 5° intervals, using a 5° diameter field of view and ar integration time of  $\tau = 2$  min to ensure a high precision in the measured position angle,  $\chi$ , of the polarisation plane. For more complete coverage of the night sky, we now observed on both the north and south meridian, at altitudes of 35.0°, 41.9° to 83.3°, and 90°, again using a 5° diameter field of view but with an integration time of only 30 s. A reduction in  $\tau$  was necessar in order to maintain the same spatial resolution—about : square degrees. For  $\tau = 30$  s, the typical uncertainty in  $\chi$  is 6°small enough to allow the detection and monitoring of significan detail in the polarisation. The possible influence of tropospheric scattering is minimised by observing above an altitude of 35 (and on the N-S meridian). We also monitored tropospheric effects by observing a fixed set of points, all with a declination of 20.7°, at various altitudes. We found that at Haleakala tropospheric scattering at 5,300 Å is usually unimportant above an altitude of 35°. Occasionally though—for instance, during enhanced airglow activity—close examination of the data fo tropospheric scattering effects may be necessary.

Polarisation maps can be prepared from the data; these arplots of the polarised intensity vector in an ASP-centred diagram (Fig. 1). Maps of Q, U and pI ( =  $[Q^2+U^2]^{1/2}$ ) can also b obtained1.

The polarisation maps show several effects: first, sufficiently far away from the ASP (and from the galactic plane) the polarisation plane tends to be normal to the scattering plandefined by the Sun, the Earth, and the zodiacal dust particle This orientation can be expected if the dust particles are spherical or non-spherical and randomly oriented.

Second, within about 30° from the ASP, there are significandeviations from this orientation. They take the form of two polarisation patterns: pattern a, with relatively small values o pI (typically less than 0.8 S<sub>10</sub> (V) near the ASP), and with nearly random orientation of the polarisation plane (Fig. 1a); pattern i

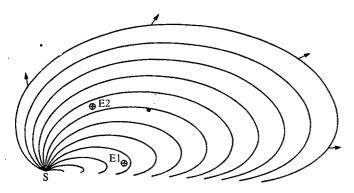


Fig. 2 Schematic representation of an expanding shock front from the solar wind. The Sun is at S, the Earth at two alternative positions, E1 and E2 (see text).

has pI values typically larger than 1.5  $S_{10}$  (V) near the ASP, with the polarisation plane tending to be normal to the ecliptic plane (Fig. 1b).

Third, a pattern usually persists for as long as a week, which was our typical useful observational period during a lunation, with only minor changes occurring in that pattern. At least one example of a sudden change was, however, observed, between August 25 and 26, 1973 (compare Fig. 1a and b).

Fourth, the influence of the Milky Way on the polarisation is seen clearly when the galactic equator crosses the ecliptic within about 30° of the ASP. There is a tendency for the polarisation vectors to be rotated normal to the galactic equator (Fig. 1c).

Fifth, the polarised intensity does not vanish at the ASP; that has already been established<sup>1</sup>.

The observed effects can be understood if the zodiacal dust is non-spherical and if, at distances from the Sun typically greater than 1 AU, it can have preferred directions of orientation, that is, be partially aligned. Observational evidence of the existence of appreciably non-spherical grains has been presented elsewhere (refs 1-3, and R. D. Wolstencroft, and J. C. Brandt, unpublished). The fact that at elongation angles,  $\epsilon$ , of less than  $90^{\circ}$  the polarisation plane tends to be normal to the scattering plane implies that close to the Sun the particles are more nearly oriented randomly. This conclusion is supported by the observations of Sparrow and Ney4, who detected no change in the zodiacal light at 90° elongation over a 4-yr period.

We suggest that the polarisation patterns, a and b (Fig. 1a and b) correspond to two states of particle orientation: an undisturbed state (Fig. 1b) in which the particles are partially aligned; and a disturbed state (Fig. 1a) corresponding to random orientation. We have suggested (ref. 1, and R. D. Wolstencroft and J. C. Brandt, unpublished) that the interaction of the dust with the solar wind plasma and with the interplanetary magnetic field, changes the orientation and the degree of alignment of the grains. Details of the theory of the interaction need to be established, particularly the way in which significant changes in grain orientation are produced sometimes in only one week, or less. Nevertheless, we can suggest models which relate the observed changes of the polarisation pattern to disturbances in the solar wind plasma of the magnetic field.

Consider the influence of the disturbed solar wind. As the shock front associated with a solar wind disturbance moves outward from the Sun and into the domain of grains which are usually aligned, an expanding region of disoriented grains forms in that domain (that is, at distances typically greater than 1 AU). Consequently, the polarisation pattern in the antisolar hemisphere changes from b to a (Fig. 1a and b). The effect of the zone of disoriented grains on the polarisation pattern depends on the position of the Earth. If the position is that of E1 in Fig. 2, evidently only part of the antisolar hemisphere shows a disturbed pattern. If the position is E2, the entire hemisphere is affected. But the angular size of the disturbed pattern also depends on the scattering function of the grains and may be

smaller than the size of the region in which the orientation of the grains has been disturbed. When the line of sight through the zone of disoriented grains becomes long enough, observable changes in the polarisation pattern will occur; it will occur a few days after the shock front has encountered the Earth. The change in pattern will be gradual and should be most noticeable close to the ASP where the particles beyond the front contribute relatively little to the polarised intensity.

Now consider the influence of regions of disordered magnetic field, which are associated with sector boundaries. As in the case of shock fronts from the solar wind we assume that the passage of the sector boundary disorients the grains. If the time needed for the particles to be re-aligned were greater than the time that occurred between boundary passages (typically one week) then the grains would be permanently disordered. The orientation of the polarisation within 30° of the ASP given by pattern B is inconsistent with permanent disorientation of the grains. When the sector boundary reaches the Earth we should see a sharp edge dividing the polarisation pattern into ordered and disordered zones (corresponding to patterns b and a, respectively). As the boundary moves past the Earth, this edge moves towards the ASP and into the morning hemisphere. For reasons related to the scattering function, already mentioned, the moving edge may not be well defined, however, and may therefore not be detected until it has come close to the ASP.

As the observed changes in polarisation patterns depend on the position of the Earth relative to the disturbances in the interplanetary medium, as well as on other factors (such as the scattering function of the dust), correlating pattern changes and events in the interplanetary medium is not a trivial task. In fact, we have not been able to attribute the sudden change between August 25 and 26, 1973, to any interplanetary event. We are not discouraged, however; if a clear correlation between pattern changes and events in the interplanetary medium were established, there would exist a unique method of observing and monitoring the interplanetary medium in regions as yet inaccessible to space probes.

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### Arithmetic of ice ages

THE Milankovitch hypothesis is that glaciations occur when the Northern Hemisphere receives relatively little summer heat from the Sun, because of astronomical factors that alter the orientation of the Earth's axis and the eccentricity of its orbit. A new time scale for the ocean-bed record of the past eight glaciations is provided<sup>1,2</sup> by the magnetic reversal at the start of the Brunhes epoch, 700,000 yr ago. In addition, Vernekar's recalculated insolation tables3 are now available. For a popular account of climatic change4, I wanted to illustrate the opportunity thus provided, for a re-evaluation of the Milankovitch hypothesis.

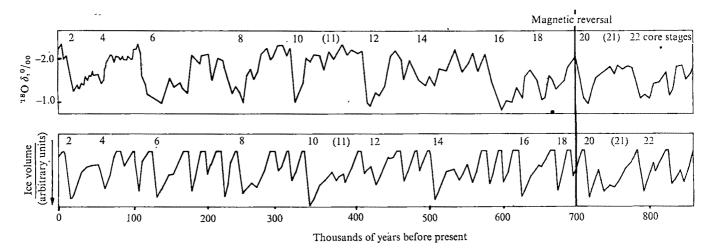


Fig. 1 Upper, measured oxygen-isotope changes in marine organisms from Fig. 9 of ref. 1; low points indicate a large volume of ice. They are plotted against depth in core and so the dating is uncertain except at the magnetic reversal 700,000 yr BP. Lower, ice volume derived by arithmetic as described in the text, from 50°N insolation data given in ref. 3 (inverted, arbitrary units).

An inspection of Vernekar's tables suggested that the crucial zone for summer sunshine was not at the Arctic Circle, as has often been supposed, but at 50°N. The theory of ice sheets growing from the bottom up<sup>5,6</sup> and the discovery of the English Channel glacier<sup>7,8</sup> fed from the seabed south of Ireland make this choice of latitude glaciologically plausible.

Suppose, now, that a decline in summer sunshine at 50°N below a certain level allows the volume of glaciers and ice sheets to grow in simple proportion to the deficit, while summer sun-

Table 1 Comparison of corrected ocean-core dates of maximum glaciation, taken from Fig. 4 of ref. 2, and the dates deduced arithmetically.

	•	
 Core stage	Ocean-core date (to nearest 5,000 yr)	Arithmetical date (to nearest 1,000 yr)
2	20	- 16
4	65	62
6	110 or 140	133
8	245	248
10	330	344
12	420	429
14	505	509
16	595	-626
18	665	676

Dates in thousands of years BP.

shine above that level melts ice with a different proportionality. For the point of equilibrium I take 17 langley per day (1 langley = 1 calorie cm $^{-2}$ ) above the 1950 value of 847 langley per day. Glacial maxima or minima are then predicted at the dates when the summer insolation at 50°N crosses the +17 level.

Simple summing, by 1,000-yr steps, of the surpluses or deficits above or below +17 indicates in arbitrary units the extent of freezing or melting between the maxima and minima. With a melting rate of unity, a freezing rate of 0.22 gives a realistic curve for the most recent glaciation (the past 78,000 yr); melting the ice is easier than making it. The same proportionalitites are then applied over the past 860,000 yr. Finally, as surplus sunshine cannot melt ice that is not present, a limiting value for the reduction in ice volume can be assumed. The running totals then predict the amount of ice in the world. Figure 1 compares the resulting arithmetical curve with measurements of the oxygen-isotope ratios in marine fossils<sup>1</sup>, which are also thought to be proportional to the amount of ice in the world. Table 1 compares the dates of maximum glaciations

arrived at arithmetically, with the corrected dates deduced by Emiliani and Shackleton<sup>2</sup> who take account, as best they can, of variations in ocean-bed sedimentation rates.

Meteorological processes are so notoriously nonlinear that my assumptions are almost frivolous. The matches between the curves of Fig. 1 and the dates of Table 1 are very much better than they deserve to be unless the Milankovitch effect is indeed dominant. The arithmetical curve captures all the major variations and the core stages can be identified with little ambiguity. The defects include an excessive warm peak 170,000 yr ago, and excessive freezingin the more recent parts of corestages 11 and 21. On the other hand, the arithmetical curve registers two known events not conspicuous in Shackleton's oxygen-isotope curve, namely the cooling at 90,000 yr ago<sup>9,10</sup> and the warming at 100,000 yr BP required by the speleothem data<sup>2</sup>.

Other processes are at work, including the 2,500-yr oscillation<sup>11</sup> that correlates with <sup>14</sup>C production in the atmosphere, and hence with solar events rather than with the Milankovitch effect. Nevertheless the variations in summer sunshine available for melting the snow of the Northern Hemisphere plainly determine the first-order pattern of past glaciations. One can even put an order of magnitude to the process. A surplus of one langley per day above the +17 level, at 50°N in summer, will melt sufficient ice to change the oxygen-isotope ratio of oceanwater at a rate of 0.01% per thousand years.

Extrapolation of the curve gives a first-order forecast (Fig. 2) for the 'next' ice age, which began 5,000 yr ago and will end 119,000 yr from now. Broecker and van Donk<sup>12</sup> arrived at a broadly similar forecast by more general reasoning from the insolation predictions. This ice age looks like a relatively slow starter. The theory, though, is of widespread snow that fails to

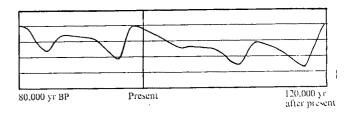


Fig. 2 Enlargement of the arithmetical curve for the most recent (Würm/Wisconsin) glaciation and an extrapolation for 120,000 yr into the future by the same arithmetical rules.

melt in the vicinity of 50°N in summer, so that large areas of North America, northern Europe and the USSR will have to be encrusted with ice sheets during the next few thousand years, to fulfil the expectations of Fig. 2.

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# New dates from the Ethiopian plateau volcanics

INTERPRETATION of geological events in Ethiopia has been severely limited by the lack of isotopic age data from the plateau volcanics. Volcanism was thought to have begun in the Eocene -an idea based chiefly on ages from the Blue Nile Gorge basalts<sup>1</sup>. These basalts have been resampled and ages of 23 to 27 Myr determined<sup>2</sup>. Recent research involving the measuring and sampling of volcanic profiles on the Western and South Eastern Ethiopian Plateaus, has made it possible to sample the basal flows which immediately overlie Mesozoic sediments. We report here nine new K-Ar dates from the Mugher, Adigrat, Garamullatta, and Jijiga profiles (Fig. 1.) The data are presented in Table 1 together with brief petrographic descriptions. All

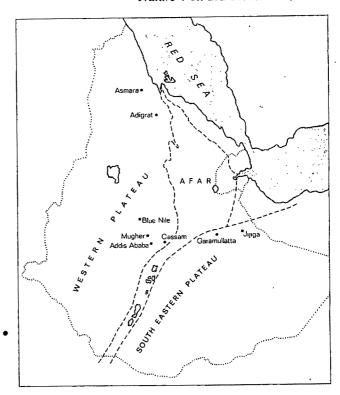


Fig. 1 Profile locality map of Ethiopia.

samples contain up to 12% intersertal glass; whole rock samples were analysed. The errors quoted are at the 60% confidence limit and based on replicate analyses.

The ages are striking both in their stratigraphic consistency and in the general similarity between profiles. Although it may be argued that they do not represent the oldest volcanics of the Ethiopian volcanic episode, nevertheless they do indicate that a considerable part of the plateau sequence is very much younger

	Table 1 Sample data										
Sample No.	Profile	Position in profile	%K	Vol. <sup>40</sup> Ar rad. s.c.c. g <sup>-1</sup> × 10 <sup>-6</sup>	%40Ar rad.	Age(Myr) Petrography					
30410	Jijiga	Second flow	0.93	0.8550	74.6	22.8±0.7. Fine-grained sparsely feldspar-phyric. Plag. (An <sub>52</sub> ), pale brown augite and abundant opaque ore. 10% clear green isotropic glass.					
30409		Basal flow	0.72	0.6840	61.0	23.6±0.7 Feldspar-phyric basalt. Plag. (An <sub>55-80</sub> Zoned) in matrix of plag. (An <sub>53</sub> ), pale brown augite and opaque ore. <5% clear glass.					
30903	Adigrat	300 m from base	0.70	0.6461	78.4	Feldspar basalt. Phenocrysts (approx. An <sub>55</sub> ) in sub-trachytic texture of plag laths, augite, and opaque ore. <1% glass.  Small clusters of slightly altered olivines in matrix as					
30888		Second flow	0.61	0.5766	57.4	23.5±0.7 below. < 5% green glass.  Medium-fine grained aphyric basalt. Ophitic texture—					
30884		Basal flow	0.62	0.7286	66.6	29.2±0.7 plag.laths (An <sub>5</sub> r) enclosed in pink titanaugite with abundant magnetite. Approx. 5% altered olivine 7-8% pale green glass.					
30940	Garamullatta	200 m from base	0.72	0.4836	49.3	16.7±0.7 Medium-fine gined feldspar basalts. Small plag. phenocrysts (Angl) in sub-trachytic matrix composed					
30945		15 m from base	0.53	0.4824	59.8	22.7±0.7 of plagioclase, pale-brown augite, fresh olivine and opaque ore. Approx. 10% pale brown, isotropic glass.					
30954	Mugher	Second flow	0.86	0.7137	65.1	20.6±0.7 Porphyritic basalt. Clinopyroxene phenocrysts and clusters in very fine sub-trachytic matrix with abundan opaque ore. 10-12% green isotropic glass.					
30948		Basal flow 1.07		0.8451	78.7	19.8±0.7 Porphyritic basalt. Clusters of twinned, zoned plagioclases, pyroxenes and rare olivines in a fine matrix of similar composition. Less abundant opaque ore. Approx. 10% greenish-brown glass.					

than originally inferred. This is in good agreement with the new Blue Nile data, quoted above, and with an age of 20.9 Myr from the Cassam sequence3.

These K-Ar data cast considerable doubt on the theory that the volcanism began in the Eocene or early Oligocene4 since none of the ages so far obtained exceeds 30 Myr.

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# Possible environmental index in tropical reef corals

PRELIMINARY results from a mineralogical study indicate that hermatypic corals may preserve in their skeletons a continuous record of noncarbonate sediments that were suspended in the waters in which they grew. Studies of coral structure, skeletal chemistry, and skeletal mineralogy1-6 have tended to neglect the significance of this trapped material. Our findings suggest that any comparison between the abundance of trace elements in corals from different areas may be questioned, as may those studies which suggest that large amounts of silica and aluminium are incorporated in skeletal carbonate.

This study was restricted to noncarbonate detrital fractions in the skeletal cavities of live, tropical reef corals which were free of macroborings filled with sediment. Specimens were from the eastern coast of the United States, the Gulf of Panama, and the barrier reef off Belize. They were prepared by coarse crushing freshcut, weighed coral fractions; dissolution at room temperature and pH 11 in ethylenediaminetetraacetic acid (EDTA)<sup>7</sup>; treatment with 30% H<sub>2</sub>O<sub>2</sub> to remove oxidisable organic material; drying and weighing of the residue; and then mounting the residue on glass slides for X-ray diffraction analyses. Mineral components were identified using standard analytical techniques8. Annual radial density bands, recorded in X radiographs<sup>9,10</sup>, were used to estimate growth increments in coral sections (Fig. 1).

Detailed microscopic examination of both freshly broken surfaces and surfaces etched with acid revealed some skeletal cavities which had been capped with a dissepiment after they had been partially filled with detrital material (Fig. 2). The dissepiments were commonly arched to accommodate trapped sediment, indicating that the polyps were alive when the sediment entered the skeletal cavities. As coral polyps can normally reject particulate material which is smaller than silt11, the sediment probably entered the skeletal cavities when the polyp was damaged or destroyed, and became sealed into the skeleton after polyp regeneration or recovery of a segment of the coral

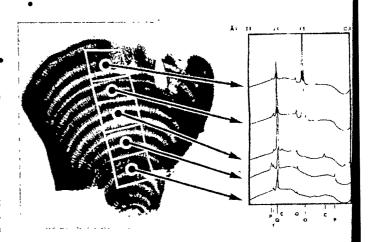


Fig. 1 X radiograph of Pavona gigantea showing variation in composition of noncarbonate detrital fraction relative to annual paired light and dark density bands. Note the appearance of gibbsite (4.85 Å) approximately six years ago. C, chlorite; T, tale; G, gibbsite; Q, quartz; F, feldspar.

colony. This type of material, however, was not found embedded in the skeletal carbonate matrix.

X-ray diffraction analysis of residue in specimens from the eastern United States showed the mineral suite: chlorite gibbsite, illite, kaolinite, montmorillonite, quartz, talc, and several feldspars (Table 1). As considerable mineralogica variation occurs in detrital material from corals of the sam species, the mineral suite is not species specific. It is, howeve related to the character of suspended or resuspended emateria

Table 1 Amount and composition of noncarbonate detrital material in corals											
Coral sample and location	Approximate range of years in the past	Amount of entrapped sediment (p.p.m. by weight)	Mineral suite*								
Solenastrea hyades (Dana) North Carolina Shelf	present-92	580	I-T-KC-G-Q-F								
Solenastrea hyades (Dana) North Carolina Shelf	present-91	103	M-I-T-KC-Q-F								
Solenastrea hyades (Dana) North Carolina Shelf	present-117	150	I-T-KC-G-Q-F								
Solenastrea hyades (Dana) South Carolina Shelf	present-50	750	I-G-Q-F								
Solenastrea hyades (Dana) Florida Bay	unknown	1,550	I-T-KC-G-Q-F								
Pavona gigantea Verrill Gulf of Panama	1–16	171	T-G-Q-F								
Montastrea annularis (Ellis and Solander) Belize	2–16	243	I-G-Q								

<sup>\*</sup>M, montmorillonite; I, illite; T, tale; KC, kaolinite-chlorite; G, gibbsite; Q, quartz; F, feldspars and plagioclases.

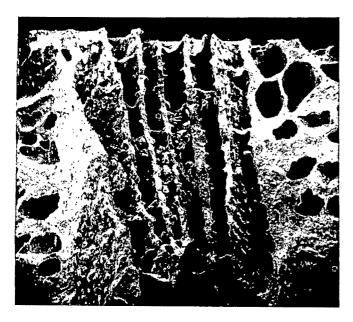


Fig. 2 Detrital material trapped in corallite of Solenastrea hyades (Dana) from North Carolina (× 16). Note dissepiment cappings.

in the surrounding water masses<sup>12-14</sup>. No evident chronological patterns were observed in the mineralogical composition of this residue.

Residues from the Panamanian and Belizean corals include gibbsite, quartz, and several feldspars. These samples show that gibbsite (Al<sub>2</sub>O<sub>3</sub>) was introduced at the same time as the total amount of residue increased. The occurrence of gibbsite in the Panamanian specimen (Fig. 1) correlates in time with the start of a housing development in the Pearl Islands (P. W. Glynn, personal communication).

The type of noncarbonate detrital material in coral skeletons, therefore, may be dependent largely on spatial and temporal variations in the composition of sediment suspended in waters in which the corals lived. Thus, major changes in the kind of residue throughout the skeleton of a coral colony are probably the result of past changes in the character of suspended detritus.

Changes in the concentration and composition of trapped -detritus should be included in baseline studies because suspended -material is part of the total environment of a coral colony. Environmental interpretations could become more meaningful if mineralogical changes of trapped, suspended material were considered together with elemental distributions in coral skeletal carbonate. Detrital aluminosilicates are associated with many ecologically important trace elements<sup>15</sup>, so that any analytical scheme must consider the possible presence of detrital minerals if the calculation of partition coefficients is to be significant. • Cechniques for the separation of this detrital material must secessarily be chemical, because of its disperse nature in primary keletal cavities. Furthermore, the techniques should be lesigned to prevent the release of adsorbed or included metal ons from the detrital minerals.

Previous elemental chemical analyses must be interpreted vith caution in cases in which the presence of terrigenous detritus was not noted. Early coral-skeletal studies concen--rated on the major elements traditionally associated with the arbonate framework (Ca, Mg). A few trace metals were also envestigated, but little real attention was paid to them until it vas demonstrated16 that oxides of Fe, Al, and Si, in addition to •nost of the trace metals found in seawater, are present in coral keletons. There have been attempts to calculate partition or Mistribution coefficients for these elements<sup>5,8</sup>. Our work, and that of others16, suggests that most, if not all, Fe, Al, and Si, along with some alkalis and alkaline earths reported in coral

heads, are attributable to detrital aluminosilicates and other detrital minerals, rather than to material incorporated in the skeletal carbonate.

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# Radiation dosage associated with ball lightning

A BALL lightning event occurred in a house in North Berkshire on May 8, 1970; here we report subsequent investigations using thermoluminescent dating techniques. According to some theories1-4 matter close to the path of such an event may experience a radiation dose of order 1 to 1,000 rad. Since the house was built of brick and only 25 yr old, it seemed possible that the mineral inclusions in the brick would exhibit a level of thermoluminescence (TL) significantly in excess of the level resulting from the annual radiation dosage received from the uranium, thorium and potassium contained as impurities in the clay of the bricks themselves.

The material tested was supplied by Ashby and Whitehead3. Cores (mostly around 30 mm in length) were taken from bricks around the doorway through which the ball lightning is reported to have passed (cl, 2, 4 and 7 of Fig. 1) and a series of controls well displaced from the event were added to the collection (c3, 10 and 12 of Fig. 1). Radioactive analysis of the clay (by alpha counting in the case of uranium and thorium, and by flame photometry in the case of potassium) indicated

that those crystalline inclusions which are free of self-radioactivity, such as quartz<sup>6</sup>, would experience an annual dosage of a little under 0.5 rad, thus accumulating TL equivalent to 12 rad since the bricks were fired.

In the measurement procedure<sup>5</sup> particular care was taken to suppress 'spurious' TL that was liable to show itself in the 275° C region of the glow curve; in addition to the utilisation of oxygen-free nitrogen, the grains were coated with silicone oil using an aerosol spray. The very low concentration of crystalline material in these bricks, ( $\sim 1$  to 2%), excluded the usual pretreatment of the sample by etching in hydrofluoric acid (this limits the sample to quartz and so ensures that the estimate of natural dose-rate is accurate). Radioactive analysis of an

Table 1 Measurements of equivalent doses in bricks from a North Berkshire house recorded as in the vicinity of a ball lightning event

		· · · · · · · · · · · · · · · · · · ·
	High temperature TL equivalent dose (rad)	Pre-dose TL equivalent dose (rad)
(i) Doorway cores		
c1 (i)	20	*
c2 (i)	16	*
c4.1 (i)	*	14
c4.2 (i)	14	*
c4.2 (ii)	16	*
c7.1 (i)	*	17
c7.1 (ii)	24	*
c7.2 (i)	19	*
c7.2 (ii)	*	15
c7.2 (iii)	*	16
(ii) Control cores		10
c3 (i)	15	*
c3 (ii)	16	*
c10 (i)	19	*
c10 (ii)	*	12
c12 (i)	*	15
c12 (ii)	*	12
, ,		

<sup>\*</sup>Since some of the crystalline extracts were small only one method of TL analysis was attempted on each sample.

extract indicated that at least some of the crystalline minerals in the sample had an internal content of uranium and thorium even if the quartz present did not. A rough value of about  $0.15\,\mathrm{rad}\,\mathrm{yr}^{-1}$  was estimated for the resulting extra annual dosage, adding a possible 3 rad to the calculated accumulated dose in the cores.

As well as the conventional high temperature TL analysis<sup>5</sup> we investigated the sensitivity changes of the 100° C quartz peak<sup>6,7</sup> under the combined effect of radiation plus heating to 500° C. A similar heating of freshly annealed geological quartz causes no sensitivity change, nor is there any change if the sample is irradiated and the TL induced allowed to decay away at room temperature (the storage halflife is about 145 min). But if the sample is heated to 500° C there is an increase in sensitivity which is proportional to the size of the 'pre-dose'-the radiation dose received between the annealing

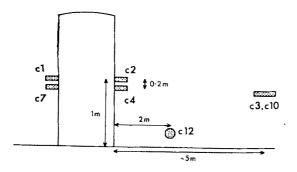


Fig. 1 Location of core samples.

and the heating to 500° C. In the present application we are concerned with the natural pre-dose that has been received by quartz in the brick between firing in 1945 and laboratory analysis in 1970.

The higher values of the estimated dose obtained by the high temperature method reflect the limitations on accuracy of this approach when only small amounts of sample are available (Table 1). The agreement between the predicted dose of 12 rad for quartz in the control cores and the observed pre-dose values (averaging at 13 rad) is satisfactory. The variations are probably due to the difficulties in estimation of the initial sensitivity values appropriate to quartz only. In the supposed 'hotter' doorway cores, though the average dose is slightly higher at 15.5 rad, the difference from the controls can hardly be treated as significant. If a ball lightning dosage occurred it would seem to be less than 5 rad in magnitude.

The model of ref. 1 would require a ball lightning event of less than 105 J at 2 m to produce such a dose and the model of ref. 3 would require an energy content of less than 2×10<sup>3</sup> J (less than  $2 \times 10^{-11}$  of antimatter).

This work should not be treated as in agreement or otherwise with another TL study<sup>8</sup> of possible effects of a ball lightning event in 1846. The author of ref. 8 was unable to detect any dosage in a damaged church steeple made of stone. Even the upper limit of 1,000 rad quoted earlier would be barely detectable in the face of the geological dose that the TL of such stone would record. We also feel that comments on the use of TL stored in the 110° C peak as an indicator of high dose radiation history should be read with the knowledge of the shortness of the peak's halflife. Even a delay of 1 d between event and TL measurement will reduce the peak intensity by a factor of around 1,000. But the sensitivity changes due to the pre-dose mechanism are stable over millennia.

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# New type of smectic mesophase?

4-Cyano-4'-octylbiphenyl (NC.C<sub>6</sub>H<sub>4</sub>.C<sub>6</sub>H<sub>4</sub>.C<sub>8</sub>H<sub>17</sub>) is a bipheny derivative of considerable technological interest since it forms colourless, stable smectic phase which exists at room tempera ture (21° C to 32.5° C). The corresponding ether 4-cyano-4 octyloxybiphenyl (NC.C<sub>6</sub>H<sub>4</sub>,C<sub>6</sub>H<sub>4</sub>,OC<sub>8</sub>H<sub>17</sub>) forms a similar smectic phase at a higher temperature range (54.5° C to 67° C Preliminary studies suggested that the smectic phases of thes compounds are not miscible with any recognised type of smect phase. Subsequent investigations (unpublished work by G.W.G have shown, however, that they are both miscible with a number of 'authentic' SA phases.

The X-ray diffraction photographs of the smectic phases c the two biphenyls are similar and are of a novel type. They bot consist of a sharp inner reflection and two diffuse outer refle tions as shown in Fig. 1. All smectic phases previously studie

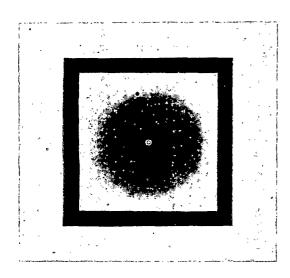


Fig. 1 The X-ray difraction pattern of 4-cyano-4'-octylbiphenyl, taken at room temperature with a flat plate camera. The sample was contained in a Lindemann glass tube (of diameter 0.1 mm). No specific measures were taken to orientate the sample and the partial ordering apparent occurred spontaneously. The innermost sharp reflection corresponds to a repeat distance of 31 Å. The two diffuse reflections correspond to ranges of repeat distances centred on 8.6 Å and 4.3 Å.

have given X-ray diffraction patterns which fall into one of the three categories (listed by de Vries² as types  $\alpha$ ,  $\beta$  and  $\gamma$ ). In all of these there are sharp inner rings corresponding to the smectic layer thickness. In type  $\alpha$  patterns there is one diffuse outer reflection, in type  $\beta$  there is one sharp outer reflection and in type  $\gamma$  there are three sharp outer reflections.

In the diffraction pattern of the biphenyls, the outer diffuse reflection corresponds to a d spacing of 4.3 Å and is at more or less the same position as the single diffuse reflection given by previously studied  $S_A$  phases (all of which are essentially conjugated aromatic systems of the general type p-X.C<sub>6</sub>H<sub>4</sub>.A = B.C<sub>6</sub>H<sub>4</sub>.Y-p). We note that the diffuse reflection characteristic

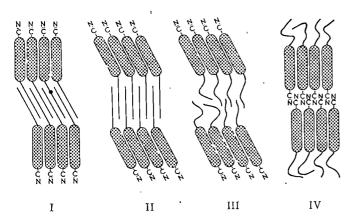


Fig. 2 Diagrammatic representations of possible alternative models for the smectic phases of cyanobiphenyl derivatives. I, Simple model featuring fully extended, interdigitated alkyl chains; II, model incorporating tilting of the biphenyl groups, which may be necessary to reduce the repulsive dipole-dipole interactions between the CN groups; III, model in which the alkyl chains are disordered and only partially interdigitated; IV, alternative model incorporating disordered alkyl chains where the two sheets of molecules in each layer are held together by the electrostatic interactions between the interdigitated CN groups in the centre of the layer.

of fluid hydrocarbon chains is found at a slightly lower angle, corresponding to a spacing of 4.5 Å or 4.6 Å, and no subsidiary maximum is discernible at this position in the diffraction pattern. The origin of the inner diffuse reflection is not apparent to us at this stage. The inner sharp reflection corresponds to a spacing of 31 Å, which is to be compared with the length of approximately 22 Å for the fully extended molecule. Some form of double layer structure is clearly mandatory. A number of possible models for the packing arrangement which have the correct layer thickness are shown diagrammatically in Fig. 2. All of these have layers of a sandwich construction. In model I the alkyl chains are fully extended and interdigitated. One questionable feature of this model is the juxtaposition of the CN groups. The bond dipole moments of these are appreciable (we would expect a value of about 4 debye units) and perhaps model II, which would reduce repulsive dipole-dipole interactions, is preferable. There is also the possibility incorporated into model III that the alkyl chains are disordered—which would reduce or remove the need for interdigitation. In the final model, IV, it is suggested that it is the interactions between the -CN groups, rather than between alkyl chains, which hold the two sheets of molecules together within the layer. It is hoped that this matter will be resolved (and the structural features which give rise to the two diffuse rings found) by our current investigations using mesophase samples oriented in a magnetic field and by extrapolating from the structures of the crystalline solids.

Optical studies by Gray and Harrison have indicated that both mesophases are uniaxial and only in the case of model IV are the individual layers uniaxial. Models I-III may, however, not be ruled out on this account because they would give uniaxial phases also if there were no correlation of the tilt directors from smectic layer to smectic layer.

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# Orientation perception by children

In a series of publications Bryant<sup>1-3</sup> has advanced the interesting idea that children's perceptual processes in the early years are characterised by an absence of absolute codes and an exclusive reliance on relative codes. Much of this work involves the perception of orientation of lines: in a successive discrimination task (that is one in which the choice stimuli are presented after the standard has been withdrawn) children below the age of 5 yr have been found able to distinguish horizontal from vertical lines but not oblique lines from their mirror-image. Bryant suggests that children adopt a binary match-mismatch code, which tells them whether lines parallel each other or not. Horizontal and vertical lines can be matched with the framework of the plaque on which they are presented and coded accordingly; oblique lines, however, merely produce a mismatch signal in such a comparison. Thus the young child cannot as yet code orientation in an absolute sense and instead requires the help of features of his environment with which the stimuli can be compared.

It follows from such a view that even oblique lines ought to be discriminated correctly by young children when the task is a simultaneous discrimination situation. In such a situation the standard is presented together with the choice cards, so that, according to the match-mismatch hypothesis, the child can code the orientation of the correct line as being parallel with the standard. In simultaneous comparisons, near-perfect performance should therefore occur, even when the discrimination involves oblique lines. Bryant's studies seem to confirm this; we have, on the other hand, not been unable to replicate his

Our data were obtained by four groups of postgraduate students in successive years between 1970 and 1973, using identical methods and thus representing four replications. The 114 subjects (mean age 4 yr 5 months) were seen individually in a room at the nursery school which they attended, and presented with 4 inch square white cards on which 3 inch long black lines were drawn. There were two stimulus conditions, involving discrimination between a horizontal and a vertical line and between two oblique lines respectively, and two test conditions, that is, simultaneous and successive discrimination. Subjects served under all four conditions, receiving eight trials under each.

An analysis of variance of the error scores obtained by the whole group shows highly significant effects for stimuli (F= 88.68, P < 0.001), for test conditions (F = 69.96, P < 0.001), and for the interaction between these two terms (F = 24.58, P <0.001). It seems that the children found the horizontal-vertical discrimination considerably easier than that between oblique lines; that they performed very much better in the simultaneous than in the successive discrimination test; and finally, and most important, that the oblique stimuli were difficult under both conditions (see Table 1).

Table 1 Mean number of errors (max. 8, chance level 4)										
Stimuli	Simultane Mean	ous task s.d.	Successive task Mean s.d.							
Horizontal-vertical Obliques	1.10 3.17	1.48 2.01	3.10 3.89	1.80 1.59						

It is thus apparent that even when the standard card and the two choice cards were presented at the same time, the children were unable to discriminate among obliques. The presence of a stimulus for matching purposes was of little help in judging similarity, whereas no such difficulty arose in the discrimination of horizontal and vertical lines. Exactly the same pattern of results emerges when each of the four sets of data obtained in different years is analysed separately.

These results thus fail to confirm those reported by Bryant. It is not clear what factors may be responsible for the discrepancy, but taken in conjunction with the failure by Fellows and Brooks<sup>4</sup> to replicate another deduction from Bryant's theory, namely that children should be able to discriminate obliques in a successive task when the lines are presented within a diamond-shaped framework, it becomes apparent that reservations are necessary in accepting the match-mismatch explanation. It seems rather more likely that children initially have an absolute difficulty in coding oblique lines that is not overcome by the provision of parallel lines in the environment.

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# Diapause potential in tropical flesh flies

FLESH flies (Diptera: Sarcophagidae) of the temperate region use the short, cool days of late summer and early autumn as a reliable cue to signal the advent of winter1,2 Flies may enter an overwintering pupal diapause in August, and the adults will not emerge until the following May2. Tropical flies, by contrast, are not exposed to a regularly occurring season of lethal temperatures, and flesh flies from Nairobi, Kenya, have been reared outside throughout the year without the occurrence of diapause (D. L. D., unpublished). Yet four species from equatorial Africa that have been examined, Sarcophaga inzi, S. monospila, Poecilometopa punctipennis and P. spilogaster, have all demonstrated the potential to enter pupal diapause. During diapause in both the temperate1,2 and tropical species, pupal development is halted at the early phaenerocephalic stage.

Colonies of experimental flies originated from wild females, and diapause experiments were carried out within the first year of laboratory rearing. Larvae from single females were reared in separate packets of beef liver2 P. spilogaster, a species collected in Nairobi (1°S), was examined most extensively.

Induction of diapause is temperature dependent. If the larvae and pupae of P. spilogaster are maintained at temperatures of 20° C or more, development proceeds without interruption (Fig. 1a). At 18° C, a few pupae enter diapause and as the temperature decreases the incidence of diapause increases. Diapause is not, however, an immediate reaction to the temperature experienced by the phaenerocephalic pupa. The transfer of young pupae to lov temperature does not result in diapause. The abilit to enter diapause is programmed during larval life. Maxi mum diapause response is obtained by cold exposure throughout larval life. A decrease in the duration o cold exposure (15° C) received by the larva decrease the incidence of diapause (Fig. 1b). If more than th first 2 d of larval life are spent at 25°C, the diapaus response is reduced to less than half the maximal res ponse. During July and August, the coldest month in Nairobi, mean daily temperatures are frequently low as 15-18°C; however, for induction of diapause series of 1-2 weeks of cool days would have to occur direct succession. An interruption by several warm day could avert the diapause. Although an accumulation of sufficient cold days for diapause induction occurs only rarely in Nairobi, at higher elevations within 20 km Nairobi, conditions that could induce diapause occu annually.

Unlike the temperate species, which react strongly photoperiod2, the tropical species seem to be uninfluence daylength for diapause induction. Differences coul

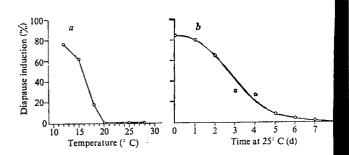


Fig. 1 a, Incidence of pupal diapause in P. spilogaster that were reared throughout larval life at different temperatures and a daily 12-h photophase. Each point represents 982-1,411 pupae. b, Incidence of pupal diapause in P. spilogaster that were held at 25° C for different periods following larvi-position and then transferred to 15° C. Each point represents 174-214 pupae.

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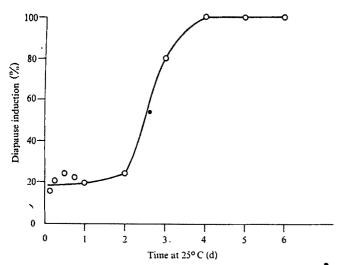


Fig. 2 Percentage of *P. spilogaster* that terminate diapause when transferred from 15° C to 25° C for different periods of time and then returned to 15° C. Pupae were in diapause 10 d before exposure to 25° C. Each point represents 25 pupae.

not be established among flies reared under daily photoperiods of 1, 10, 11, 12, 12.25, 12.50, 13, 15 and 24 h. Since seasonal variation in daylength is only 7 min in Nairobi, the results are not surprising.

Termination of diapause is temperature dependent in both temperate<sup>2</sup> and tropical flesh flies; spontaneous termination of diapause occurs later at lower temperatures. At 18°C, diapause in P. spilogaster lasts 72.6  $\pm$  2.9 d (mean  $\pm$  s.e., n=108), at 15° C 100.9  $\pm$  2.2 d (n=268), and at 12° C 234.6  $\pm$  6.8 d (n=207). Flies from the two regions, however, do differ greatly in their rate of response to favourable temperatures. If young diapause pupae of a species from a temperate region are transferred to 25°C, more than 100 d may elapse before diapause is terminated2. If diapause pupae of P. spilogaster are transferred from 15° C to 25° C, adult development can be observed within 3 d. The amount of exposure to 25° C required for diapause termination for P. spilogaster is seen in Fig. 2. In this experiment, pupae were exposed to 25° C for varying periods of time and were → then returned to 15° C; 4 d at 25° C was sufficient for diapause termination in all pupae. The difference in the ability to terminate diapause between flies of the two regions is a reflection of the environmental conditions to which each is exposed. The temperate region winter is a predictable event of long duration. The tropical seasons are less predictable. Years may go by without the species entering diapause. If the fly does enter diapause during a brief cold period, it is not committed to a long period of dormancy and can react immediately to a stimulus of higher temperatures.

That diapause has been observed in all four Sarcophaginae examined suggests that the potential for diahause may be common among the tropical species. The Sarcophaginae are thought to have originated in the ropics and subsequently spread to the temperate regions3. The necessity for diapause increases with distance away rom the equator, and the temperature dependency of tiapause becomes supplemented with photoperiodic cues <n the temperate region.

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# A fly's leap from paralysis

THE first temperature-sensitive paralytic mutant of Drosophila melanogaster, parats, was isolated four years ago1-3. The mutation was mapped as a point at 53.9 on the X chromosome<sup>4</sup>. The present day stock of parats moves normally at 22° C, but is paralysed within 10 s at 29.5° C. Paralysis is usually complete. The legs are held close to the body causing the fly to lie on its back or side. When returned to 22°C the fly recovers its mobility within 5 s. A much slower and less complete recovery occurs when the flies are left at 29.5° C (ref. 4). Only after 30 min are they able to walk and after 2 h a few flies can be induced to fly by shaking their container. The mutation has been shown to act in a regionally autonomous fashion within the head and thorax5.

By genetic and pharmacological means, activity can be elicited from parats flies at temperatures which would otherwise paralyse them. Augmented inhibition is suggested as a possible cause for this type of paralysis.

Our work concerns the interaction of para's with Hyperkinetic<sup>1</sup> (Hk<sup>1</sup>), another sex-linked mutation and the pertinent behaviour of this mutant is its response to movements nearby<sup>6</sup>. When waved at by hand it usually jumps and often tumbles. In our laboratory, Patrick Wong discovered that the fly responds similarly to a high intensity, high frequency stroboscopic light7. High speed motion picture films show that the take off and flight are abnormal8.

While hand waving seems to elicit a few jumps from Hk1 para ts flies at 22°C, these double mutants do not respond to this stimulus after they have been paralysed at 29.5° C. Hk1 parats flies, however, usually respond to the strobe light at 22° C and a few jumps can even be elicited after they have been paralysed at 29.5° C.

Hk1 parats flies did not respond under the high levels of heat and light emitted by the lamps used for high speed photography. Fortunately, flies carrying mutations which produce yellow cuticle (y) and white eyes (w) (ref. 9) together with Hk1 and parats (that is, y w Hk1 parats) were very responsive. Figure 1 is a photographic sequence of a y w Hk1 parats fly responding to a flashing light at 29.5° C. The first frame shows the paralysed fly lying on its right side facing away from the camera. After the flash, the fly pushes off with the midlegs and beats its wings twice before leaving the field of view. Other sequences show as many as seven wing beats. Normally Hk1 parats and y w Hk1 parats flies recover their mobility over a prolonged period, just as parats flies do. Yet at each instant from the time that paralysis is first induced, these flies retain the competence to see and to respond.

The light-induced escape from paralysis is short lived. The fly travels 2 or 3 cm and falls down in a paralysed state again. Furthermore, the response is often incomplete. The fly may push off with its midlegs, simultaneously lifting and spreading its wings only to return its wings to a resting position in midflight. The extension of the midlegs may also be incomplete: the femurs extending while the tibias remain folded. Such blocks in the jump and flight response have not been seen in wild type or Hyperkinetic flies and are therefore probably caused by the para's mutation.

Table 1 shows that the capacity of the  $Hk^1$  mutation to make the parats mutant jump at high temperature is greatly enhanced by the w mutation<sup>10</sup>. w Hk<sup>1</sup> para<sup>ts</sup> flies respond to both flashing

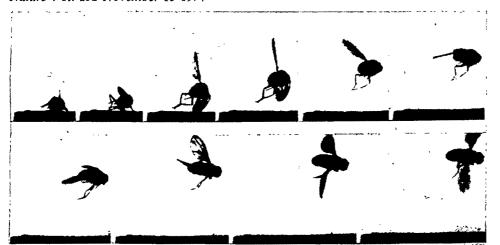


Fig. 1 The response of a y w Hk<sup>1</sup> para<sup>ts</sup>/Y fly to flashing light at 33° C. The sequence was recorded at 400 frames s<sup>-1</sup> in 5,500 lux illumination. 100 light flashes s<sup>-1</sup> were delivered by a Grass PS2 photostimulator at intensity setting '8'. The PST2 lamp and reflector were placed 20 cm from the fly. Paralysis was complete before stimulation.

light and movements. There is no indication that the mobility of  $w Hk^1 para^{ts}$  flies is greater than that of  $para^{ts}$  flies at elevated temperatures. Rather the added mutations seem only to sensitise the flies to visual stimuli.

Obviously, the sensory, neuronal and muscular elements involved in seeing, jumping and flying are functional. A degree of motor competence in paralysed para<sup>ts</sup> flies has also been demonstrated previously by the production of leg jerks through electrical stimulation of the cervical connectives<sup>11</sup>. On the other hand, the temperature-induced paralysis may be caused by abnormally high levels of inhibition of elements innervated by inhibitory neurones. When the light reaching the sensory cells is increased by the w mutation<sup>12</sup>, and the motor system is genetically primed by the Hyperkinetic mutation, certain visual stimuli elicit strong signals which override inhibition and call forth the high priority response of escape<sup>8</sup>.

Picrotoxin specifically blocks the action of  $\gamma$ -aminobutyric acid<sup>13</sup>, a probable inhibitory transmitter in insects<sup>14–16</sup>. Thus it may also prevent temperature-induced paralysis from occurring in  $para^{ts}$  flies.

Table 1 Average response of all mutant combinations of y, w,  $Hk^{1}$  and  $para^{ts}$  to hand waving and flashing light

	Number of jump responses $\pm$ s.d. to 50 stimuli 22 $\pm$ 1° C 29.5 $\pm$ 0.5° C								
				29.5 ± 0.5° C hand light					
Genotimo	hand		ght	hand					
Genotype	waving	'4'	sities '8'	waving	inten:	'8'			
y w Hk1 parats	22+10	$\frac{32+12}{32+12}$	-	17+13	$\frac{4}{36+11}$	27±12			
+ w Hk1 parats	31 + 17		19 + 11	20±19					
$+ + Hk^{i}$ para <sup>ts</sup>	$3\pm 4$	$23 \pm 13$	$32 \pm 14$	0+1	3+4	5+6			
$+ + Hk^{1} +$	$41\pm7$	$22 \pm 13$	29±11	35±9	<b>3</b> ∓3	$3\pm3$			
$+ w Hk^1 +$	$43\pm3$	$47 \pm 3$	$47 \pm 2$	$48\pm 2$	49±2	$48\pm 2$			
$y w Hk^1 +$	$35 \pm 11$	44±7	24±13	$47\pm3$	$46 \pm 4$	$47 \pm 3$			
yw++	0 + 0	0 + 0	0+0	0 + 0	0+0	0+0			
y+++	0+1	$6\pm7$	$12 \pm 12$	0 + 0	0 + 0	0+0			
+w++	0+0	0 + 0	0+0	0 + 0	0+0	0+0			
$+++para^{ts}$	$1\pm1$	5±6	$8\pm9$	0 + 0	0+0	0+0			
$y + + para^{ts}$	0+0	$10 \pm 12$	9±11	0 + 0	0+0	0+0			
$+ w + para^{ts}$	0+0	5±7	$1\pm 2$	0 + 0	0 + 0	0 + 0			
$y + Hk^1 +$	$45 \pm 6$	$39 \pm 11$	$40 \pm 12$	$36 \pm 14$	28±19	$22 \pm 14$			
$y + Hk^1 para^{ts}$	$5 \pm 10$	$36 \pm 15$	$39 \pm 15$	0 + 0	$3\pm6$	5±5			
$y w + para^{ts}$	0 + 0		0+1	0 + 0	0 + 0	0 + 0			
<del>**</del> + + *	0+0	0+0	0+1	0 + 0	0+0	0+0			
(Canton-S)									
<del>*</del> + + <del>*</del> *	0+1	0+0	0+0	0 + 0	0+0	0 + 0			
(Oregon-R)									

Ten males, 4–5 d old, of each genotype, were placed individually into 8 cm  $\times$  2 cm vials, which were then laid on a white background. Each fly was subjected to quick horizontal passes of the hand about 5 cm above the vial, or light stimuli, each consisting of 10 flashes of 0.01 s duration, produced by a Grass PS2 photostimulator. At settings '4' and '8', the peak luminous intensities of the xenon lamp are stated to be about 2.2  $\times$  10's and 2.4  $\times$  10's candlepower. The lamp and reflector were placed 15 cm above the vial. Ambient intensities of illumination from overhead fluorescent fixtures were 700 lux at 22  $\pm$  1° C and 350 lux at 29.5  $\pm$  0.5° C.

Injections of picrotoxin elicited a random activity in para<sup>15</sup> and wild type flies at 22° C. Low concentrations caused the normal activities of the flies to be carried out in a shakey, uncoordinated manner. Higher concentrations increased the activity of the fly and abolished all coordination. The behaviour of all flies was not identical, but in various individuals, movements of the head, antennae, proboscis, wings, halteres,

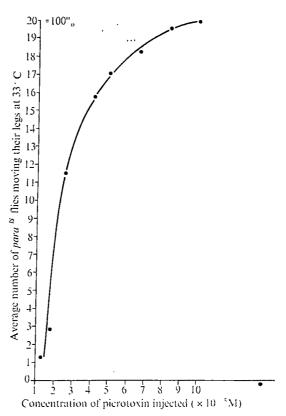


Fig. 2 Effect of picrotoxin on leg movements of para's flies at 33° C. Male and female para's flies, 3-24 h old, reared at 17° C were immobilised on a cold plate!8 and injected in the ventral posterior abdomen with about 0.25 µl of picrotoxin in Drosophila Ringer's¹º or Drosophila Ringer's alone. The dissecting scope lights were fitted with small water chambers to reduce heat. Uninjected control flies were kept cold for the same period. Two lots of 10 injected and 10 uninjected flies were placed in four covered thin plastic dishes²º. At 22° C they recovered from the cold until at least 80% of the uninjected flies were able to stand (4-6 min). The dishes were then transferred to a water heated observation chamber. The interior of each dish rose from 22° C to 33° C within 220 s. From 250-600 s, the number of flies able to move even a leg segment was noted at 50 s intervals. Other types of movements were not monitored. The average number of flies moving their legs is recorded on the graph. Over 90% of uninjected and Ringer's injected flies lay in a state of paralysis. In the same conditions, wild type Oregon-R flies were not paralysed, but did tend to stand still, walking and preening infrequently.

abdomen and legs were seen. We assume that these activities were the result of a blanket disinhibition of many parts of the nervous system which generate motor activity. These uncoordinated movements of parats also occurred, albeit less vigorously, at 33° C. The number of flies able to move their legs increased with increasing concentrations of the picrotoxin injected (Fig. 2).

In our experiments, the concentration of picrotoxin reaching the central and peripheral nervous system are expected to fall within the range which has been shown to specifically block GABA-mediated peripheral inhibition ( $5 \times 10^{-6}$  M to  $4 \times 10^{-5}$ M) (ref. 13). Other physiological effects which have been reported<sup>17</sup> in the  $5 \times 10^{-5}$  M to  $10^{-2}$  M range are unlikely to account for the behaviour we observed.

Picrotoxin injections caused as many as 8 deaths in a group of 20 treated flies. Flies which survived recovered their normal behaviour on the day following treatment.

The motor elements of the paralysed fly seem to be functional but the possibility that paralysis may be caused by the reduced excitability of certain motor elements cannot be ruled out. In these circumstances, normal levels of inhibition may cause paralysis, while the blocking of normal inhibition may have caused the movements that we observed. On the other hand, augmented inhibition still provides a reasonable explanation for a paralysis which can be overridden or disinhibited.

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# Origin of Nicotiana tabacum L. detected by polypeptide composition of Fraction I protein

THERE is no well-authenticated record of the occurrence of Nicotiana tabacum, the commercial tobacco plant, in the wild state, and so its origin and evolution are of great interest. N. tabacum (n=24) is believed to have arisen by chromosome doubling after hybridisation of N. sylvestris Spegazzini and Comes (n=12) with a species in the Tomentosae section of Nicotiana. Goodspeed and Clausen¹ suggested that N. tomentosa Ruiz and Pavon (n=12) was the species, but Clausen<sup>2</sup> amended it to N. tomentosiformis Goodspeed (n=12). Goodspeed<sup>3</sup> favoured N. otophora Grisebach (n=12), however, because of its present-day geographical distribution; N. otophora is found together with N. sylvestris in an area on the eastern slopes of the Andes whereas N. tomentosiformis occurs further north where N. sylvestris has not been found. Later evidence, however, suggests N. tomentosiformis as the more likely progenitor of N. tabacum. Gerstel<sup>4</sup>, from an analysis of the segregation of artificial polyploids of N. tabacum × N. tomentosiformis and N. tabacum $\times$  N. otophora, concluded that N. tomentosiformis showed the greater chromosome homology with N. tabacum, and Sheen<sup>5-7</sup> from an analysis of isoenzyme patterns and sterol composition supported the conclusion that N. sylvestris and N. tomentosiformis were the likely progenitors of N. tabacum. From an analysis by isoelectric focusing of the polypeptide composition of Fraction I protein isolated from N. tabacum and the putative progenitor species, we now report that N. tabacum arose from the hybridisation of N. sylvestris  $\mathcal{P} \times$ N. tomentosiformis 3.

Fraction I protein (ribulose 1,5-diphosphate carboxylase) consists of two kinds of subunits which differ in size. The large subunits are coded by chloroplast DNA8 whereas the small subunits are coded by nuclear DNA9. By isoelectric focusing in 8 M urea the large subunit is resolved into three polypeptides and the small subunit into one or more polypeptides<sup>10</sup>. The patterns obtained are characteristic of each species of Nicotiana; no variation of pattern has been observed within any species although many plants from several seed collections have been examined. The polypeptide patterns for Fraction I



Fig. 1 Polypeptide composition of Fraction I proteins from N. tabacum and the putative progenitor species. Crystalline Fraction I protein was dissolved in 8 M urea-0.5 M Tris-HCl, pH 8.5 and reduced by the addition of dithiothreitol. The protein was S-carboxymethylated by the addition of iodoacetic acid, and after 15 min excess iodoacetic acid was removed by passage through a 1×7 cm column of Sephadex G-25 (coarse). Protein (20 µg per sample) was then applied to a prefocused 4.5% polyacrylamide gel slab containing 1% Ampholine, pH 5-7, and 8 M urea. Isoelectric focusing was performed for 18 h at 300V. Polypeptide bands were stained with bromophenol blue. Minor bands marked by arrows are artefacts arising by carbamylation of the protein by cyanate present in urea solutions<sup>10</sup>. Samples, from left to right, were N. otophora, N. tomentosiformis, N. tabacum and N. sylvestris. Large subunit polypeptides, pH 6.5; small subunit polypeptides, pH 6.0.



Fig. 2 Polypeptide composition of Fraction I proteins. Fraction I proteins were S-carboxymethylated and subjected to isoelectric focusing as described for Fig. 1. Arrows indicate artefacts due to carbamylation of the protein. Band splitting is due to the reaction of polyphenols with the protein during isolation (J.C.G., S.D.K. and S.G.W., unpublished results). All samples were 20  $\mu$ g protein. Samples, from left to right, were N. sylvestris  $\times$  N. otophora (n=24), N. sylvestris, N. tabacum, N. sylvestris  $\times$  N. tomentosiformis (n=24).

protein isolated from seven different cultivars and a polyploid series of N. tabacum were all similar (K. Chen, S.D.K., J.C.G. and S.G.W., unpublished results). Isoelectric focusing of Fraction I protein therefore readily provides phenotypic markers for both the nuclear and chloroplast genomes11.

Crystalline Fraction I protein was prepared from young leaves by a simple method<sup>12</sup>. Modification of the homogenisation buffer was necessary to obtain crystals from N. tomentosiformis and N. otophora; Dowex 1-( $\times$ 2) (20% w/v), KCN (1 mM), sodium metabisulphite (10 mM) and bovine serum albumin (0.1% w/v) were included in the homogenisation buffer to prevent interaction of the protein with polyphenols18. The Fraction I proteins were recrystallised three times before analysis of the polypeptide compositions by isoelectric focusing.

The polypeptide compositions of Fraction I proteins isolated from N. tabacum and its putative progenitor species are shown in Fig. 1. The protein from N. tabacum is composed of three polypeptides in the large subunit and two polypeptides in the small subunit. Comparison with the polypeptide compositions of Fraction I protein from the other three species indicates that N. sylvestris contributed the large subunit polypeptides and therefore was the maternal parent in the original hybridisation. N. sylvestris also contributed one of the two small subunit polypeptides of N. tabacum, the other polypeptide being contributed by N. tomentosiformis. This analysis indicates that the original hybridisation was N. sylvestris  $9 \times N$ . tomentosiformis 3 and that N. otophora was not involved in the origin of N. tabacum.

To further verify this conclusion Fraction I proteins were isolated from experimentally-produced hybrid plants and the polypeptide compositions were analysed by isoelectric focusing. As shown in Fig. 2, the polypeptide composition of Fraction I protein isolated from the amphidiploid (n=24) of N. sylvestris  $\times N$ . tomentosiformis is exactly the same as the composition of the protein from N. tabacum, whereas the Fraction I protein from the amphidiploid (n=24) of N. sylvestris  $\times$  N. otophora has a different small subunit composition from the protein from N. tabacum. The amphidiploid of N. sylvestris  $\times N$ . tomentosiformis is taxonomically indistinguishable from N. tabacum and is readily crossed with tobacco cultivars.

The analysis of Fraction I proteins therefore provides a convenient means for determining the origin of plant species that have arisen by interspecific hybridisation. The particular advantage in the use of Fraction I protein is that, because the large subunit polypeptides are inherited solely from the maternal parent, it is possible to determine the exact parenta, in the original hybridisation.

The multiple polypeptide compositions of the large at small subunits of Fraction I proteins have also been demostrated in the proteins isolated by conventional chromatograph methods from spinach (Spinacia oleracea), soybean (Glyci. max) and barley (Hordeum vulgare) (K. Chen, S.D.K., J.C.( and S.G.W., unpublished results). It therefore seems probab that the method used with the Nicotiana species can be applie to studies of the origin of species in other plant families.

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# Lithium treatment reduces morphine self-administration in addict rats

RECENT reports suggest that opiate-seeking behaviour i. experimentally-addicted animals can be reduced by administer ing other drugs, such as α-methyl-p-tyrosine and haloperidol1-4 and that haloperidol can also alleviate symptoms of heroir withdrawal in man<sup>5</sup>. Both drugs have been shown to reduc morphine-induced hyperactivity in mice6.

Lithium also has been shown to interact with morphine: ; can sometimes reduce morphine-induced hyperactivity is mice7,8 and potentiate morphine analgesia in rats9. We have therefore investigated the possibility that lithium may affec the amount of voluntary ingestion of morphine by addict rats

Naive rats normally reject solutions of morphine, presumabl because of their bitter taste, but with a suitable drinkin schedule they eventually learn to consume appreciable amounts<sup>10,11</sup>, especially if the morphine is given in solution of sucrose12.

Male hooded rats, weighing 180-200 g at the beginning of the experiment and singly housed, were first trained to satisfy their daily fluid requirements during a limited time (1200h-1500h

with food being available *ad libitum*. We have shown that rats readily adapt to this schedule and maintain apparent good health for at least 12 months<sup>13</sup>.

The rats were then made dependent on morphine by being given bottles containing solutions of 10% w/v sucrose +0.5mg ml<sup>-1</sup> morphine hydrochloride (SM) as their only source of fluid. Within a few days they learned to drink more of this solution than they had previously drunk of water. To test the strength of their preference for SM the animals were, after 6 weeks of this forced SM drinking, given daily choices between two bottles which contained either SM or a solution of 10% sucrose alone (S). On average the rats drank some 57% of their total fluid intake in the form of SM. There were considerable differences among them but individual rats were remarkably consistent over long periods of time. (Three out of the seventeen rats were excluded from the experiment as they persistently rejected the SM; nevertheless the statistical comparisons reported here remained significant even with the three rats included in the analyses.)

Fluid consumption was measured throughout by weighing the drinking bottles and applying a correction for spillage in the course of placing and removing bottles from the cages. The positions of the bottles (right or left) were varied randomly each day to allow for possible positional preferences. Statistical comparisons are based on two-tailed *t* tests and on analyses of variance designed to take into account repeated measures on the same subjects<sup>14</sup>.

After 3 weeks of daily SM/S choices, the rats were prepared for lithium administration by being weighed and injected intraperitoneally with 13 ml kg<sup>-1</sup> of isotonic saline 3 h before the beginning of their daily drinking period for 2 weeks. Then half the rats were injected intraperitoneally with 2 mmol kg<sup>-1</sup> of an isotonic LiCl solution and half continued being injected with saline. The particular dose of LiCl was chosen because our previous experiments had shown it to be behaviourally effective and without manifest toxic effects<sup>15</sup>, whilst yielding plasma levels approximating the lower end of the therapeutic range in man<sup>16</sup>. As lithium efflux from the brain is rather slow it is likely that our schedule of injections was adequate for maintaining fairly constant brain Li levels<sup>17</sup>.

After the very first injection of LiCl, the rats reduced their normal intake of SM from 23 ml d<sup>-1</sup> to 15 ml d<sup>-1</sup>, that is, by approximately 35%, and increased the intake of S by 47% from 15 to 22 ml d<sup>-1</sup> (P < 0.05). This pattern of drinking

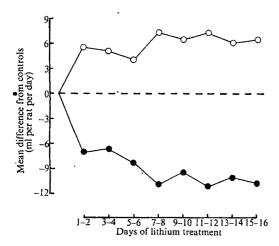


Fig. 1 Addicted rats which were given daily choices between solutions of,  $\bullet$ , 10% sucrose + 0.5 mg ml<sup>-1</sup> morphine hydrochloride (sucrose-morphine) and,  $\bigcirc$ , 10% sucrose alone (sucrose), were injected daily with either 13 ml kg<sup>-1</sup> of isotonic saline or with 2 mmol kg<sup>-1</sup> of LiCl (n=7 per group). Lithiumtreated rats reduced their consumption of sucrose-morphine from 23 ml d<sup>-1</sup> to an average of 15 ml d<sup>-1</sup>, saline controls (---) increased theirs slightly. The results are represented as mean differences from controls.

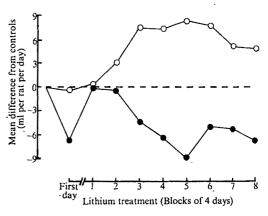


Fig. 2 Addicted rats which were given daily choices between sucrose-morphine ( $\bullet$ ) (Fig. 1) and tap water ( $\bigcirc$ ) were injected with 2 mmol kg<sup>-1</sup> of isotonic LiCl (n=9) or 13 ml kg<sup>-1</sup> of saline (n=8). Lithium treated rats reduced their sucrose-morphine consumption consistently only after some 12 days. The results are presented as differences between LiCl and saline controls (---).

•persisted relatively unchanged throughout the 16 d of lithium treatment (Fig. 1). Lithium-treated rats consistently drank less SM ( $F = 10.5 \, d.f. = 1$  and 12, P < 0.01) and more S (F = 5.5, P < 0.05).

The possibility could not be excluded that these changes in the relative amounts of the two fluids consumed might have been the result merely of an interaction of lithium with taste, that is, lithium (injected intraperitoneally) could have increased the bitterness (and thus the aversiveness) of the SM, or it could have increased the relative palatibility of the S. Therefore, in a second experiment, 24 rats, after being accustomed to satisfying their thirst during the 3 h periods each day, were divided randomly into two groups. Twelve rats were given solutions of 10% sucrose+0.025% quinine hydrochloride<sup>11</sup> (SQ) and twelve 10% sucrose solution alone as their sole source of fluid for 5 weeks. This concentration of quinine proved, initially, to be as aversive as the SM at the beginning of the previous experiment. When the SQ rats were subsequently given daily choices between SQ and S, they persistently drank some 40% of their daily fluid intake in the form of SQ. The remaining twelve rats which had been given sucrose alone to drink continued to receive S only. All rats were then handled and injected with saline for 2 weeks, and as in the previous experiment, they were divided into equal groups which continued to be injected with saline or were given 2 mmol kg<sup>-1</sup> isotonic LiCl for 2 d. Lithium treatment had no effect on the amounts of either SQ or S consumed, regardless of whether the rats had a choice between SQ and S, or were given S alone.

These results suggest, therefore, that the reductions in the amount of morphine ingested during lithium in the SM experiment were unlikely to have resulted merely from changes in the palatibility of the fluids, as in the SQ experiment lithium did not alter the consumption of either S or SQ.

To analyse the lithium-morphine interactions further, an experiment was devised in which the rats would voluntarily consume much greater amounts of morphine (44 mg kg<sup>-1</sup> d<sup>-1</sup>) than in the original experiment (27 mg kg<sup>-1</sup>, P < 0.001). This was achieved by training 17 rats to drink SM in a way similar to that used in the first experiment, but in this instance the rats were given a choice between SM and plain tap water. They consequently drank more than 90% of their total fluid requirements in the form of SM. When, at the appropriate stage of the experiment, they were injected with 2 mmol kg-1 LiCl their SM consumption was reduced (F = 29.7, d.f. 1 and 15, P <0.01) and their consumption of water increased (F = 29.7,P < 0.001); however, in these rats the reduction of morphine intake became consistent only after some 12 d (Fig. 2), possibly because the degree of their dependence was based on a much higher dose of morphine.

It seems fair to conclude that we have clearly demonstrated a robust interaction between morphine and lithium substantial reductions of morphine self-administration were obtained in two different experiments. Should further research establish the generality of these results, their possible implications for clinical practice seem obvious.

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# **Experience modifies morphine-induced** behavioural excitation of mice

In many animal species, relatively low doses of morphine produce not only analgesia but also a state of behavioural excitation, restlessness and increased locomotor activity which has been defined as 'running fit'1. A large intraspecific variability in locomotor activity has been recorded after the same dose of opiates2 and clear strain differences have also been reported. Morphine for example, has been shown to enhance the locomotor activity of C57BL/6J mice and to drastically reduce it in the DBA/2J strain3.

In man a number of studies indicate that the mechanisms responsible for individual differences in the reactivity to different psychotropic agents such as tranquillisers or hallucinogens are under genetic control<sup>4,5</sup>. A number of environmental, social and psychological factors, however, may also play a role in the determination of the effects of many psychotropic agents such as psychedelic drugs or opiates6 and it is generally acknowledged that the social environment influences the action of centrally acting drugs, These factors have been scarcely studied in animals but a number of experiments demonstrate that drug action is affected by group interactions or by the environment. In particular, Steinberg and her associates, investigating maze exploratory behaviour in individual rats, have shown that

past experience can have a very marked effect on, or even abolish the increase in, activity which normally results from different drugs or drug mixtures.

Our studies were designed to see if the stimulating effects of morphine on behaviour are determined, in addition to genetic mechanisms, also by environmental factors. C57BL/6J mice were used in this experiment as this strain was shown to be particularly reactive to the stimulating effects of morphine on locomotor activity as measured by a procedure previously described10. We first tested the effects of a single morphine injection on the motor activity of eight naive mice during a 25 min session. Morphine-HCl was injected intraperitoneally at a dose of 20 mg kg<sup>-1</sup> which was found to exert a clear effect on motor behaviour in this strain<sup>3</sup>. A threefold average increase was evident in the morphine-injected group as related to the activity evident in eight other naive mice injected with saline (Table 1; t=16.8, P<0.001). To test the effects of past experience on the action of morphine another group of rhice was placed for 25 min in the toggle-floor boxes and immediately after the end of this session the mice were

Table 1 Effects of morphine on the locomotor activity of naive and experienced mice

Treatment	and activity c	ounts ±s.e.	during	1st and 2nd	i sessions
Group	1st se	ssion		2nd se	ssion
1	Saline	$59.1 \pm 3.6$			-
2	Morphine	$161.4 \pm 9.1$			-
3		$51.8 \pm 3.0$		Saline	$41.5 \pm 2.9$
4	Saline	$56.5\pm4.0$		Morphine	$45.3 \pm 3.5$
				•	

Each session was 25 min long. Groups 1 to 4 were injected with saline or morphine (20 mg kg<sup>-1</sup>) immediately before the first session. Immediately after the end of the first session groups 3 and 4 were injected again with saline or morphine and tested again. Each group consisted of eight mice.

injected with morphine and tested again for 25 min. The performance during the morphine session was compared with that of a control group subjected to a similar schedule and injected with saline. Table 1 shows that past experience modifies the effects of morphine since no stimulating effect was evident in these conditions in relation to the control group (t=0.36, P>0.05).

Next we designed another schedule of experience in which the animals were placed for 25 min in the togglefloor box in the absence of drug. The mice were subjected to a second 25 min test 6 h later and at the end of this session they were injected with saline or morphine and immediately retested during a third 25 min session. By using this procedure the animals were more 'experienced' than in the previous schedule and the injection of morphine not only did not produce a stimulating effect but also resulted in a complete block of their activity (Fig. 1. groups A and B). Visual observation of the behaviour of the mice did not reveal any type of circling or other stereotyped behaviour.

At the end of this session the animals were removed from the toggle-floor boxes and returned to their home pens in which they were caged in groups of eight. When returned to their home cages the morphine-injected mice became very excited and exhibited immediately a very high leve of locomotor behaviour. After a few minutes of exploration of their home cage they were returned to the toggle-floor boxes where they exhibited extremely high levels of locomotor behaviour during a 25 min session (Fig. 1 groups A and B, session 4). Thus, exploration of a different environment was followed by the 'running fit' in the same 'experienced' animals in which the injection of morphine previously resulted in a block of their activity. Being in a group of eight (instead of being isolated in the togglefloor boxes) might have been another reason that contributed to the arousal of the mice. This result was replicated in two additional experiments.

It was possible to induce the 'running fit' in the experienced animals in which morphine resulted in a block of their locomotor activity by leaving the mice in the togglefloor boxes and switching off the 40 W lamp which was normally illuminating the sound-insulated cabin 1 m above the eight toggle-floor boxes (Fig. 1, group C). Similarly, in another group of animals the action of morphine turned from a blocking to a stimulating effect if a solution with a strong smell of eugenol was sprayed inside the cabin at the end of the morphine session. Thus, experience of the

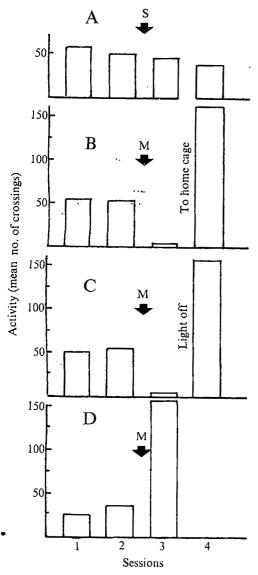


Fig. 1 Effect of past experience on the morphine-induced 'running fit'. Groups A to D were subjected to two 25 min sessions (1 and 2) spaced 6 h apart. Animals of group D were subjected to cortical spreading depression during these two sessions. Immediately after session 2 the mice were injected with saline (S) or morphine (M) (20 mg kg<sup>-1</sup>) and tested again for 25 min (session 3). At the end of this session the mice were either returned to their home cage for 2-3 min and then tested again for 25 min (session 4) or they were subjected to an additional session in fotal darkness. The performance of groups A and B during the third and fourth sessions was significantly different (t=13.1, P < 0.001; t=17.0, P < 0.001). Similarly, the performance of group C during session 4 was different from that of a control group (not shown) injected with solitons and the statements. (not shown) injected with saline and subjected to a similar schedule (t=14.9, P<0.001). In the mice tested under spreading depression during sessions 1 and 2 morphine significantly enhanced the performance in relation to that of another group of animals injected with saline (t=17.9, P < 0.001).

environment results in a suppression of the morphineinduced running fit or even in a complete block of the animal's activity while a change of the environmental conditions (such as returning the mice to their home cage, a change from light to darkness, or an olfactory stimulus) is followed by the appearence of the 'running fit' effect.

To show that experience of the environment results in a clear change of the behavioural effects of morphine we subjected a group of mice to functional decortication11 while they were subjected to two 25 min sessions in the toggle-floor boxes. In these mice the injection of morphine after the second session resulted in a clear excitatory effect (Fig. 1, group D). Also this finding shows the importance that sensory inputs and experience exert in modulating the effects of the opiate.

While further experiments are necessary to assess why the effects of morphine not only are abolished but result in a suppression of exploratory behaviour in the experienced mice, our findings indicate the importance of the environment in influencing the action of centrally acting drugs<sup>12</sup>. Study of these factors in animals might provide a useful tool for analysing the type of effects of morphine and of a number of 'social drugs' in man depending on his social environment.

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# Cervical somato-sensory evoked responses in man

It has been known for over 20 yr that cerebral responses evoked by peripheral nerve stimulation can be recorded from the scalp in man and that they may be altered by disease. But clinical application has been impeded by the variability of the cortical response, particularly of its later components, and by lack of knowledge of its origins. We have explored the possibility of recording from lower levels of the central sensory pathway.

Here we report a response which can be recorded over the back of the neck and base of the skull, which appears to be of nervous origin and has stable properties. It is likely to be useful in the study of neurological disease, notably in conditions such as multiple sclerosis where central conduction may be interrupted or delayed.

Somato-sensory evoked responses (SER) have been recorded

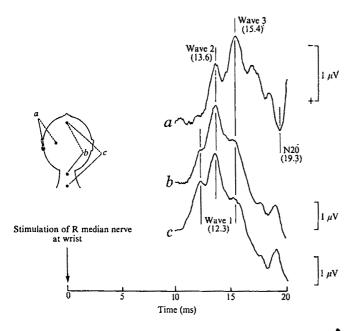


Fig. 1 Cervical somato-sensory responses evoked from a normal 20 yr old female and averaged from 256 stimuli. Electrode placement for ear lobe-parietal region (a) selectively emphasises wave 3. b and c are responses recorded from electrode sites used routinely (C2 and C7).

from surface electrodes over the cervical spinal cord in 30 normal subjects. The stimulus was a square-wave pulse of 0.3 ms duration applied to the median nerve at the wrist. Conventional surface recording and signal averaging techniques were used. Silver disk electrodes were placed between the sixth and seventh cervical spines (C7) and immediately rostral to the second cervical spine (C2) with a common mid-frontal reference electrode (Fz, 10–20 system). The cortical response from the hand area of the contralateral parietal region was simultaneously recorded. The amplifiers had a flat frequency response from 2 to 1,000 Hz. The overall response of the system was 3 dB down at 1,500 Hz, rolling off at 24 dB octave<sup>-1</sup> thereafter. The averager sampling rate was 10 points ms<sup>-1</sup> and 256 responses were summed.

Preliminary experiments showed that with increasing stimulus strength there was an initial steep rise in amplitude of response and a moderate reduction in latency, both measurements levelling off at stimulus currents approximately three times sensory threshold. A stimulus of approximately four times sensory threshold was therefore used in routine recordings. This produced a tolerable muscle twitch and was not painful. The responses from finger stimulation did not differ from those evoked from the wrist apart from the expected increase in latency and reduction in amplitude. Variation in stimulus rate between 1–10 s<sup>-1</sup> produced only minor alterations in the shape of the cervical response, in contrast to the marked changes observed in the cortical response.

With standardised stimulation at a rate of 1 s<sup>-1</sup> a predominantly negative wave of complex shape was recorded from all subjects (Fig. 1b and c). The mean amplitude (onset to peak) was 3.0 µV (range 1.4-4.9). The onset latency ranged from 9.4-11.8 ms and peak latency from 11.9-14.3 ms, both measurements being strongly correlated with arm length (Fig. 2). The mean duration was 7.0 ms (range 5.7-8.3). The cervical response was almost completed before the onset of the first component of the cortical SER at about 16 ms. Although there was some variation between individuals, three distinct peaks could be recognised within the cervical SER in all subjects. The major peak (wave 2) was preceded by about 1.3 ms by a minor peak (wave 1) and followed by a further minor peak (wave 3) after a similar interval.

This response is unlikely to be of muscular origin. In relaxed subjects its latency, amplitude and shape were remarkably constant when recorded on different occasions and changes in latency and amplitude with changing stimulus intensity closely paralleled those of the first major component of the cortical SER (N20-P28), which is accepted as neurogenic. Activity in the neck muscles due to anxiety or voluntary contraction obliterated the response whereas a myogenic response would have been enhanced. Goff, et al. recently recorded, but did not characterise, a wave of similar latency (N14) over the cervical spine evoked by stimulation at the wrist and presented convincing evidence that it was neurogenic.

Cracco<sup>2</sup> recorded from surface electrodes at various sites over the spinal cord following peripheral nerve stimulation and concluded from changes in the onset latency that the evoked response was a travelling wave. We found no such increase in latency between the lower and upper neck. The peak latencies of all three components were identical when recorded from caudal and rostral sites over the cervical spinal cord and there was no consistent change in the less sharply defined onset latency. The relative amplitudes of the three components showed systematic variation with recording site, that of wave 1 being always greatest at C7, falling off both rostrally and caudally. The amplitude of the main peak (wave 2) was similar when recorded at C7 and C2. Wave 3 could be emphasised selectively by recording from mastoid or earlobe. Some of these variations are illustrated in Fig. 1. The findings with regard to both latency and amplitude are more consistent with generation of the components of the cervical response from fixed sites than with a travelling wave in the dorsal column system. The possible locations of these sites, which may include dorsal root ganglia, spinal cord interneurones, dorsal column nuclei and cerebellum, have not yet been elucidated. There appears to have been no relevant comparison of surface with direct recordings in the experimental animal.

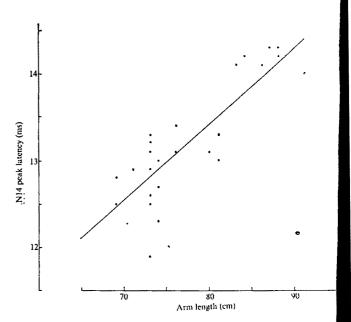


Fig. 2 Relationship between peak latency of cervical response evoked by median nerve stimulation at wrist and arm length measured from wrist to seventh cervical spine (correlation coefficient 0.85).

Surface recordings, in contrast to recordings from the epidural space<sup>3</sup>, are readily repeatable in normal subjects and patients with neurological disease. Work is now in progres designed to clarify the sites of origin of the components of the cervical SER and their use in the study of disease of the central nervous system.

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# Luteinising hormone and testosterone in man

In mammals, including man, specific sexual stimuli can affect the secretion of luteinising hormone (LH) from the pituitary and testosterone from the testis. For example, the presence of the female and copulation is associated with an increase in the blood levels of testosterone in rabbit', rat', bull<sup>3</sup> and man<sup>4</sup>, and in each species excluding man this has been shown to be related to an elevation of blood plasma LH<sup>1,3,5</sup>. Among animals, merely the presence of a receptive female may induce these changes in the absence of intercourse2,3,8 indicating that in some species visual and olfactory cues alone are sufficient to elicit an endocrine response. In man the anticipation of female company after long periods of abstinence apparently has similar effects<sup>6</sup>. Very few studies of this sort have looked at both LH and testosterone secretion simultaneously in sufficient detail to provide convincing evidence of short term endocrine changes related to behaviour. The aim of my study was to establish whether the normal pulsatile secretion of LH and testosterone in men could be altered by a specific sexual stimulus. It was decided to expose male volunteers to a film sequence which included scenes of human behaviour which were judged to be sexually provocative.

Eight healthy men aged between 24 and 29 yr were recruited. Blood samples were collected at intervals of 10-40 min using an indwelling needle inserted into a forearm vein. The trials lasted 6-7 h starting at about 1700h and only two or three subjects were studied in any one evening.

After a meal each subject spent the evening relaxing, reading and talking. The film projector was placed in a separate room and each person could view the film in complete isolation, and was free to study and relate to the scenes as he wished. None of the volunteers knew at what time they would be called to the room; they were informed at the beginning of the trial that the film may or may not contain scenes of sexual interest, and later they each completed a questionnaire indicating their reactions during the evening.

Seven out of eight subjects recorded the film sequences as sexually stimulating to a greater or lesser degree, and experienced full or partial erection of the penis at this time. The feeling of sexual excitement persisted in these subjects ■t the end of the viewing period.

There were no changes in blood levels of LH or testoterone in the subjects which could be definitely related to he films (Fig. 1). Plasma LH concentrations changed -narkedly during each trial period; intermittent high values were followed in many cases by progressively decreasing alues producing a pattern indicative of the documented sulsatile release of gonadotrophins from the pituitary<sup>7,8</sup>. Whese discharges occurred at irregular intervals; two out of ight subjects produced an LH surge during the viewing seriod which had a frequency not greater than expected in n unstimulated situation (LH surge in a resting situation

every 2-4 h would produce one or two releases in any 30 min period in eight subjects). One noticeable feature was that LH values were generally high in all subjects at the beginning of the trial, which seemed to be related to the experience of the initial vein puncture.

Testosterone concentrations fluctuated during the evening in a normal pattern<sup>8-10</sup> unrelated to the film period. By relating the testosterone values to the main LH peak for each individual and calculating a mean steroid level for the eight studies, it was possible to demonstrate a temporal relationship between LH and testosterone concentrations in the serial samples (Fig. 2). An endogenous surge in LH was associated with a progressive rise in testosterone levels which were at a maximum (+26% of value at LH surge) at 90 min after the increased LH stimulus. There was considerable variation, however, in the timing and extent of the testis response in the different cases.

I conclude that normal male subjects can experience varying degrees of sexual excitement without any concomitant change in blood LH and testosterone levels. Very few studies on man have revealed short term endocrine changes related to behaviour. Fox et al.4, studying a single individual claimed to have found significantly elevated •plasma testosterone levels during and within 5 min of coitus, and similar findings are indicated by Steinbeck<sup>11</sup>. Yet there is no evidence that these steroid changes are induced by elevated gonadotrophin levels<sup>4,12</sup>. Indeed, the present study illustrates that the human testis is relatively, sluggish in its response to endogenous changes in LH, and it can be assumed that any sexual stimulus affecting LH would not

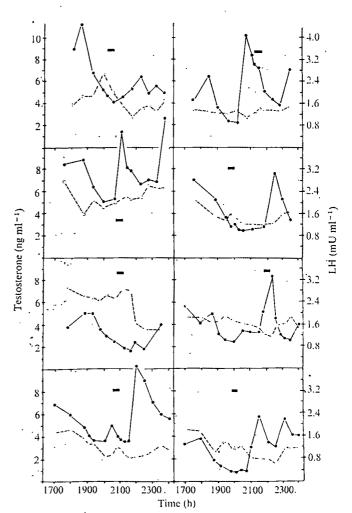


Fig. 1 Blood plasma levels of LH (•) and testosterone (()) measured by radioimmunoassay<sup>10,18</sup> in eight normal men for a 7 h evening period during which they viewed a short sexually stimulating film. ——, Film.

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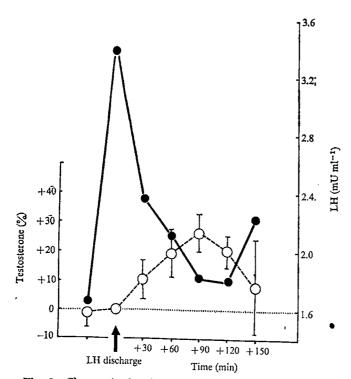


Fig. 2 Changes in the plasma concentration of testosterone in the eight subjects (mean ± s.e.) related to the principle LH peak for the individual studies. A temporal relationship between LH (•) and testosterone (○) is apparent, with testosterone values increasing after the LH discharge and reaching a maximum 90 min later.

show itself in terms of changes in plasma testosterone for some 30-90 min. It seems probable that the observations of Fox and Steinbeck may be accounted for in terms of increased blood flow through the testis which is likely to be important in causing a transitory increase in testosterone<sup>13</sup>. Neural pathways involving the hypothalamus need not be implicated in this response.

While no report has yet described short term changes in LH secretion in man related to sexual stimuli, there still remains the observation that LH levels in my study were increased at the beginning of each trial period. This involved the very first blood sample in several cases and it seems likely that the experience of having the blood sampling needle fitted into the arm may have caused this response; the LH surge occurred at the time of the venapuncture or during the hour afterwards. Studies on 'stress' associated with surgery<sup>1</sup> and army training<sup>15</sup>, as well as foot shock in rats16 and fighting in monkeys17 lead one to expect decreased LH secretion under these conditions. The observation remains, however, as a cautionary note to those using venapuncture to study gonadotrophin levels and it seems possible that a single skin puncture has a greater impact on the hypothalamic/pituitary axis than a 30 min sexually stimulating film.

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# Muscular dystrophy and muscle cell death in normal foetal development

ALTHOUGH the application of modern biochemical, morpho logical and experimental techniques to the study of musc disease has greatly extended our knowledge of myopathi processes, the cause of muscular dystrophy continues to elud investigators. McComas et al.1 have suggested that the mote neurones rather than the skeletal muscle fibres are primari affected but this has been seriously questioned<sup>2,3</sup>. My own hypo thesis is based on the concept of embryonic cell death ar stems from observations that I, as well as others, have made on the death of muscle cells in normal human foetuses<sup>4,5</sup>.

Cell death in embryogenesis takes place in a highly predic able manner and all the evidence points to it being genetical controlled. It is a familiar process in eliminating tissues an organs useful only during embryonic life, in the elimination of phylogenetic vestiges and in modelling processes such the formation of clefts or digits. The death of embryonic ce in a tissue such as muscle, where the elimination of cells appear on the face of it, to have no useful purpose, must neverthele serve a function which is probably crucial to the subseque healthy development and growth of that tissue.

I wish to suggest that the basic defect in muscular dystropt is due to a derangement in the normal process of muscle c death during a critical stage in development—possibly between weeks 10 and 16 of foetal life. From previous observations have made in human foetuses, muscle cell death can be see up to about week 16: this is roughly in agreement with Boyd observations4.5.

The derangement in the cell death process might come abo in one of three ways. First, the cell death 'switch' may simp not be activated at the critical stage in muscle developmen As a result the developing muscle fibres do not go through the proper embryological processes (of which cell death is part) and which are essential for the healthy development the tissue. The imperfectly formed muscle fibres would th die off prematurely. Second, the cell death switch may not activated at the correct time in the lifespan of the individu Instead of occurring between weeks 10 and 16 of foetal 1 it is delayed until later in development, or even into the po natal period. On this basis muscular dystrophy can be look upon as a normal process but one which has occurred too la to perform its role in development. Third, the cell dea mechanism may be triggered off at the correct time but t failure is one where the process cannot be turned offmuscle cell death would continue in a relentless fashion un

irtually all the muscle fibres had died. Here again muscular Aystrophy may be thought of as a normal process but one thich continues beyond the period when it is able to play ny useful role. One way in which this could come about ould be if the target cell (muscle fibre) contained a blocking mechanism which in some way failed to neutralise the effects f the agent responsible for the cell death.

Relatively little is known about the way in which cells die suring embryogenesis but it is known that hormones (for rample oestrogens, androgens or thyroxine) are involved in ertain specific instances. It has been shown that in amphibia lyroxine can break down the gills, tail and larval head muscles, hilst in organ cultures of tails of Xenopus the addition of popropriate concentrations of thyroxine causes regression of et tissues. Other possible mechanisms of cell death include me senescence of cells in which excessive amounts of membranous material accumulate until the point is reached when the etabolism of the cell is impaired. Errors in the metabolism of proteins which build up in the cytoplasm and block further all respiration is another possibility. In the case of embryonic all death the elimination of cells is an extremely rapid one and it is difficult to accept that cell senescence or a build up in monormal proteins could act sufficiently quickly. On the other and a genetic mutation or the production of metabolite which recifically blocks cell respiration could cause very rapid death recells. Such a specific blocking metabolite might act in a mewhat analogous fashion to the injection of certain fluoro impounds into laboratory animals, which causes very rapid apairment of cell function and structure by blocking the cidative metabolism of cells in a highly specific manner<sup>8</sup>. Morphological studies provide no clue as to the mechanism f cell death, but in the regression of tails of Xenopus larvae e death of muscle cells is accompanied by phagocytosis and auch lysosomal enzyme activity9. Although in humans the

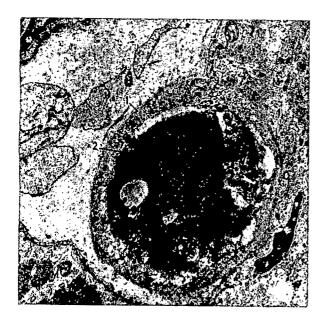


Fig. 1 Foetal muscle at 12 weeks. Quadriceps muscle was obtained from apparently normal human foetuses of either sex fresh from therapeutic abortions. Cubes of tissue (1 mm<sup>3</sup>) were fixed in 3% buffered glutaraldehyde at 4°C (pH 7.3) for th. This was followed by washing in buffered sucrose, postixation in OsO<sub>4</sub>, dehydration and embedding in araldite. Thin sections were stained on copper grids with uranyl acetate and lead citrate. The tissue was examined with an AEI EM6B electron microscope. To eliminate the possibility of traumatic «trtefact in four cases an entire lower limb was fixed immediately n 4% formaldehyde in buffered saline at 4° C and left for 24 h. small pieces of quadriceps were then post-fixed in 2% OsO<sub>4</sub> and processed as before. (1) Phagocytic cell containing ingested cell products (2), with identifiable nuclear remnant (3), M healthy myotubes. (×10,000).

degenerating foetal muscle may be surrounded by mononuclear mesenchymal cells, phagocytosis does not seem to take place on any significant scale<sup>4</sup>. In a recently examined 12-week-old foetus, however, I have observed the apparent ingestion of degenerate cell products in skeletal muscle (Fig. 1).

If, as I am suggesting, the muscular dystrophies are primarily disorders of muscle fibres, why, it may be asked, are some muscle groups severely affected while others are spared, or at any rate affected to a much lesser extent? My hypothesis goes some way to explaining this in that there is evidence to suggest that cell death is extensive in some muscle groups but is less marked or hardly occurs at all in others5.

In my view, to discover insights into the cause of muscular dystrophy, we should look to normal developmental processes in human foetal muscle and in particular the phenomenon of muscle cell death. I have suggested ways in which an upset in this phenomenon might lead to muscular dystrophy, so that these diseases can be looked upon as a normal process but one occurring at the wrong time in the individual's life span or else one which has not been repressed. Further studies of muscle cell death in normal foetuses and the foetuses of mothers who are carriers of the dystrophy gene are needed.

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# Liver as major organ of phenol detoxication?

THE ability to detoxicate and eliminate potentially harmful substances from the body represents a defence mechanism, the importance of which is well established. It has been assumed that the protective devices themselves, the detoxication mechanisms, have evolved to protect the organism from potentially harmful chemicals occurring naturally in the environment. These chemicals, usually described as foreign, are rendered harmless by metabolic transformations and modified compounds are subsequently excreted usually in the urine or bile. The liver is generally regarded as the major organ of detoxication.

The work we report here, on the fate of phenol in the rat, necessitates a reappraisal of some hitherto accepted aspects of detoxication. In particular, it focuses attention on the need to re-assess the role of the liver in detoxication.

Whole-body autoradiograms1 were prepared using young rats (approximately 50 g body weight) which had received U<sup>14</sup>C-phenol (10 μCi; 20-40 mCi mmol<sup>-1</sup>) either orally or intraperitoneally. The autoradiograms obtained U<sup>14</sup>C-phenol was administered by either route showed that the level of isotope in areas corresponding to the liver did not, at any time, exceed that of the blood. Thus, the liver has no ability to concentrate either phenol or its metabolites suggesting that U14C-phenol either fails to enter the liver cells or alternatively, that the radioactive molecules have a transient cellular existence due to rapid turnover in the liver. The qualitative

patterns do suggest, however, that the liver is not the principal site of phenol detoxication. Experiments were therefore designed to investigate further the contribution of the liver and determine the sites and extent of extrahepatic detoxication. As phenolic substances are normally presented to the animal through the gastrointestinal tract, initial studies were made to determine the detoxication capacity of the gastrointestinal tract with respect to phenol.

Isolated rat gut preparations were perfused2 with Krebs-Henscheit buffer pH 7.0 containing glucose (500 mg per 100 ml). Segments (30-50 cm) of small intestine were washed free from debris by perfusing with 50 ml of the perfusion medium before attaching to the apparatus. U14C-Phenol was added to the mucosal medium and samples were withdrawn from the circulating serosal and mucosal media over 2 h. Analysis of these samples showed a decrease in the level of radioactivity in the mucosal circulation and a concomitant increase in the level of radioactivity in the serosal circulation. When either  $U^{14}C$ -phenol (10  $\mu Ci$ ) or 10 mg of phenol mixed with U<sup>14</sup>C-phenol (10 μCi) was added to the mucosal medium 50% and 78% respectively of the radioactivity was transferred to the serosal circulation in 2 h. At the end of the experiments the radioactivity in both media was distributed between phenyl sulphate (5%) and phenyl glucuronide (95%). No unchanged U14C-phenol could be detected.

Conjugation of phenol by gut preparations in vitro raises the possibility that the gut is the principal site of detoxication of phenol ingested in the diet and that phenol is transported into the blood after conjugation. This was explored in the whole animal by perfusing the small intestine in situ with media containing U14C-phenol and simultaneously sampling blood directly from the hepatic portal vein.

Wholly anaesthetised rats (phenobarbitone) with bile-duct catheters were prepared and the gut was ligated both at the duodenal-jejunal flexure and at a point about 30 cm distal to the flexure. After washing, to remove intestinal debris, the isolated gut segment was perfused (3 ml min<sup>-1</sup>) in situ with water (30 ml) and the perfusate returned to a mixing reservoir for recirculation. Bile was allowed to join the perfusate circulation at the mixing reservoir.

U <sup>14</sup>C-Phenol (10 μCi) or 10 mg phenol mixed with U 14C-phenol (10 μCi) was added to the circulating perfusate and samples (1 ml) were withdrawn at 30 min intervals over 3 h. Portal blood samples (0.5 ml) were collected through the pancreatico-duodenal vein at 30 min intervals into heparinised tubes and the plasma was removed after centrifuging. In both experiments there was a progressive decrease in the amount of radioactivity in the circulating perfusate. Over the 3 h experimental period the major proportion of the radioactivity was transported from the intestinal lumen and was accompanied by the appearance of radioactivity in portal blood. In all plasma samples, conjugates of U 14C-phenol only were found; U 14C-phenol itself could not be detected. Phenol conjugates appeared in the intestinal perfusate within 30 min but at this time the majority of the radioactivity was still present as unchanged U 14C-phenol. In contrast, after 2 h perfusion, unchanged U 14C-phenol could not be detected and the radioactivity was present as phenyl sulphate (72%) and phenyl glucoronide (28%).

These results strongly support the view that free phenol is not transported as such from the intestinal lumen but in conjugated form. It therefore follows that the role of the liver is minimal in the detoxication of orally ingested phenol. When the gastrointestinal tract is by-passed by intravenous administration of free phenol, however, conjugation with sulphate and glucuronide and subsequent excretion of these conjugates in the urine nevertheless takes place3. In these conditions the liver may assume major importance as a centre of detoxication. This possibility was explored in experiments in which phenol was administered to hepatectomised rats.

Rats were anaesthetised with phenobarbitone and the ureters were cannulated. In test (hepatectomised) rats, hepatic

tissue (60%), the spleen and the gut were removed following appropriate ligations. When U 14C-phenol was administered intravenously to hepatectomised and control rats at dose level of 5 mg kg<sup>-1</sup> and 10 mg kg<sup>-1</sup> body weight the percentage o radioactivity recovered in urine of test and control animals ove 3 h was essentially the same. Furthermore, test and contro urines contained the conjugates of phenol although the glucuronic acid conjugate was proportionally higher in the tes than in control urines. Thus, even in the absence of the live and gastrointestinal tract, formation and urinary secretio of phenol detoxication products still occurs.

Collectively, the results strongly suggest that the detoxicatio aspect of liver function has been over-emphasised for it is show that the liver is not essential to the detoxication of pheno Attention is drawn to the considerable capacity of the gastro intestinal tract to detoxicate phenol. The findings of th investigation point to a major role for the gastrointestina tract in the protection of the whole organism from the potentia toxic effects of phenol. The necessity for such strategically placed detoxication devices is immediately apparent when it recalled that up to 600 mg of phenolic material may be ingeste each day in the normal human diet. The experiments reporte here, demonstrate that, in the case of phenol, this requiremen is satisfied by the intestinal barrier. Other potential detoxicatic sites are thus not presented with exogenous phenol since enters the portal blood in conjugated form only. The important of this latter observation is emphasised by the known effects. phenol on the red blood cells and, in retrospect, it is clear the the transport of phenol in unconjugated form by the bloc represents a highly undesirable and potentially hazardor process with respect to the health of the whole organism.

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# Cardiotoxic protein from edible mushrooms

THE cardiotoxic protein volvatoxin has been isolated from edible mushroom, Volvariella volvaceae<sup>1</sup>. We have now isola another cardiotoxic protein from the edible mushroom Flcmulina velutipes (Curt. ex Fries) Sing., which is widely eain the Orient and is canned for local consumption and expo We have called this protein flammutoxin.

Flammulina velutipes fruits throughout the year. Its c initially ovoid and yellow becomes, within one or two da flat and yellowish brown with an edge of lighter colour. I cap reaches a diameter of 2 cm; the stem achieves a length 15 cm and a diameter of 0.5 cm. This mushroom has li taste but a slightly unpleasant smell.

The method of isolation of flammutoxin was similar that of volvatoxin1 except that flammutoxin was obtain between 0 and 60% saturation of ammonium sulphate.

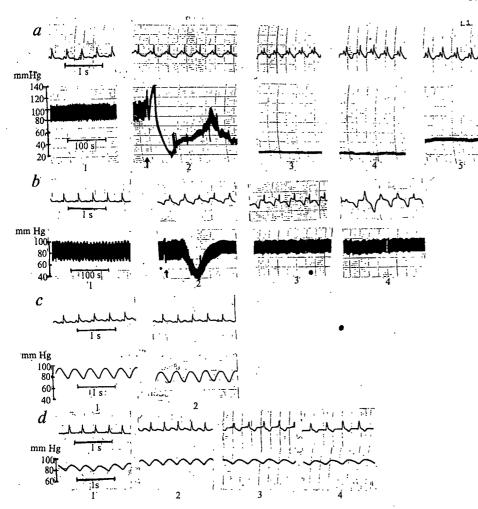


Fig. 1 Effect of a, flammutoxin and b-d, volvatoxin on the electrocardiogram and arterial blood pressure of a 2 kg cat, pentabarbital anaes-thetised (30 mg per kg body weight intraperit-oneally). Upper tracing ECG (lead II); lower tracing, arterial | blood pressure. a, 0.25 mg per kg body weight flammutoxin was injected. 1, 0 time; 2, 1 min; 3, 10 min; 4, 25 min; 5, 60 min; Arrow indicates the time of injection. b, Injection of 1 mg per kg body weight volvatoxin A2; weight volvatokin A2, 1, 0 time; 2, 1 min; 3, 10 min; 4, 60 min. c, Injection of 5 mg per kg body weight volvatoxin A1; 1, 0 time; 2, 60 min. d, Injection of 2 mg per kg body weight the mixed solution of volvatoxin A1 and A2 (weight ratio 1:2). 1, 0 time; 2, 3 min; 3, 5 min; 4, 10 min.

oncentration of flammutoxin in the mushroom was 320 mg g<sup>-1</sup> whereas that of volvatoxin A was 1,100 mg kg<sup>-1</sup>. The xicity of flammutoxin when administered intraperitoneally as determined as described previously<sup>1</sup>. The LD<sub>50</sub> was found be 2.45 mg per kg body weight. The toxicity can be comtetely eliminated by heating a solution of flammutoxin at 100° C or 20 min. Flammutoxin has three biological activities similar those volvatoxin: (1) direct haemolytic action against human oup O blood cells at a concentration of 25 µg ml<sup>-1</sup>; (2) willy to cause a writhing reaction with a delay before onset a dose of 1.2–2 mg kg<sup>-1</sup> body weight and twenty-two writhg reactions within 10 min of the first writhing reaction;

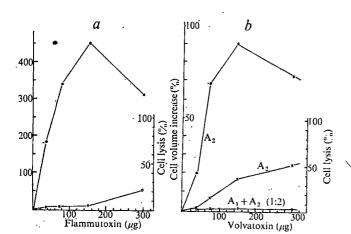


Fig. 2 Effect of a, flammutoxin and b, volvatoxin on the swelling of Ehrlich ascites tumour cells. The tumour cells  $(2 \times 10^8 \text{ ml}^{-1})$  were treated with various concentrations of flammutoxin. The reaction mixture was incubated at  $37^{\circ}$  C for 5 min and it was centrifuged at 2,300g for 30 min at 4° C with a Wintrobe tube<sup>5</sup>.  $\bullet$ , Cell volume;  $\bigcirc$ , cell lysis.

Table 1	Amino acid composition of flammutoxin		
Amino acid	Residues per 10,813		
Lysine Histidine Arginine *Tryptophan Aspartic acid Threonine Serine Glutamic acid Proline Glycine Alanine Valine Leucine Tyrosine Phenylalaning	4.10 (4) 8.15 (8) 4.02 (4) 6.88 (7) 4.02 (4) 5.72 (6) 3.88 (4)		
Total	96		

<sup>\*</sup> Measured by alkaline hydrolysis method<sup>6</sup>.

(3) effect on the ECG at a dose of 0.25 mg per kg body weight, causing depression of the ST segment and inversion of the T wave (Fig. 1a).

Flammutoxin also has several activities which were not found in volvatoxin A. First, flammutoxin induces a sharp fall in blood pressure (Fig. 1a). Second, it causes the swelling of Ehrlich ascites tumour cells (Fig. 2a). Third, it inhibits the respiration of Ehrlich ascites tumour cells (Fig. 3).

The probability that flammutoxin, like volvatoxin, might consist of two components was tested by gel filtration<sup>2</sup> and SDS acrylamide gel electrophoresis<sup>3</sup>. Flammutoxin was found to contain only one type of molecule with a molecular weight of 22,000. Table 1 shows its amino acid composition<sup>4</sup> which is similar to that of volvatoxin A2. The molecule is rich in lysine;

its isoelectric point is 4.2. The two components of volvatoxin A, A1 and A2, were tested to see whether either of them separately possessed those properties found in flammutoxin but not in volvatoxin A. Volvatoxin A2 was found to cause a decrease of blood pressure (Fig. 1b), lysis of human group O red blood cells, swelling of tumour cells (Fig. 2b), as well as the inhibition of respiration (Fig. 3) of Ehrlich ascites tumour cells. Volvatoxin A2 alone cannot however elicit the writhing reaction.

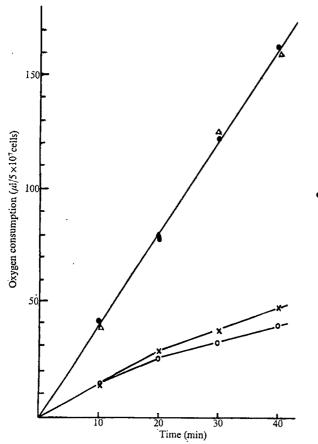


Fig. 3 Effect of flammutoxin on the respiration of Ehrlich ascites tumour cells. One millilitre of Ehrlich ascites tumour cells ( $5\times10^7\,\mathrm{ml^{-1}}$ ) was incubated with flammutoxin and the oxygen consumption was measured by manometric method with a Warburg apparatus (B. Braun Apparatebau, Melsungen, West Germany).  $\bullet$ , Control;  $\times$ , volvatoxin A2 ( $50\,\mu\mathrm{g}\,\mathrm{ml^{-1}}$ );  $\bigcirc$ , flammutoxin ( $20\,\mu\mathrm{g}\,\mathrm{ml^{-1}}$ );  $\triangle$ , volvatoxin A1 ( $20\,\mu\mathrm{g}\,\mathrm{ml^{-1}}$ ) plus volvatoxin A2 ( $50\,\mu\mathrm{g}\,\mathrm{ml^{-1}}$ ).

The activities of volvatoxin A2 were completely abolished by the presence of volvatoxin A1 at weight ratios of volvatoxin A1 to A2 higher than 1:2 (Figs 1a, 2b, 3). Volvatoxin A1 has none of biological activities shown either by volvatoxin A or volvatoxin A2. We tested whether volvatoxin A1 could affect the biological activities of flammutoxin as it affected volvatoxin A2, but we found that it had no effect on flammutoxin.

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# Dopamine 3-O-sulphate, an end product of L-dopa metabolism in Parkinson patients

Previous studies from these laboratories¹ have shown that L-3,4-dihydroxyphenylalanine (L-dopa) does not undergo sulphoconjugation in vitro in the cytosol sulphating system of rat tissues whereas dopamine very readily yields a mixture of the 3- and 4-O-sulphates. These observations might therefore serve to explain earlier claims² that sulphoconjugated dopamine constituted a significant proportion of the amine fraction extractable from rat tissues following the administration of \*\*C-labelled L-dopa and raises the question of the role of sulphoconjugation in dopamine metabolism. In man this may be of significance in L-dopa therapy in the treatment of Parkinson's disease since such conjugates might represent either intermediates or end products of metabolism.

The administration of <sup>14</sup>C-labelled L-dopa to normal people is followed by the excretion of sulphoconjugates of dopamine in the urine<sup>3</sup> and Parkinson patients on high oral doses of L-dopa have been shown to excrete up to 80% of the amine in this form <sup>4-8</sup>. It therefore seems that sulphoconjugation plays a significant role in the elimination of dopamine from the system.

In all of these studies, however, no definite proof of the absolute identity of the excretion products has been obtained It has generally been concluded that the 4-O-sulphoconjugate is excreted but in view of our earlier findings that in vitro in ra liver preparations, the 3-O-sulphate is the principal product of sulphoconjugation, such a conclusion might seem incorrect Furthermore we have shown (W.N.J., and F.A.R., unpublished that in the rat the 3-O-sulphate is metabolically inert. It does not undergo desulphation when incubated under a variety of conditions with preparations of the three arylsulphohydrolas enzymes from rat tissues and when administered intra peritoneally or intravenously to rats it is rapidly excreted in the urine unchanged. In these experiments the 4-O-sulphate behaved somewhat similarly although in contrast it underwen appreciable metabolism in vivo before excretion. Attempt have therefore been made to determine the identity of dopamin sulphoconjugates excreted by Parkinson patients on high doses of L-dopa. Urine samples (24 h) were collected from patients attending an out-patients clinic and were stored a -20° C over 2 g of sodium metabisulphite as preservative Before use the thawed urine was filtered through Whatma No. 1 paper to remove any sediment and a 50 ml aliquot of the filtrate was lyophilised after the addition of 20 µg each authentic dopamine 3-O and 4-O-35S-sulphate (prepared described previously<sup>1</sup>, specific activity ≈ 1 µCi mg<sup>-1</sup>) in 2 π of water. The addition of isotopically labelled conjugates aide in locating the relative positions of the excreted metabolite and eventually in determining the completeness of their r covery. The freeze-dried sample was then dissolved in 10 n of water and applied to a column (1.4 $\times$ 35 cm) of Dowex 1 $\times$ ion exchange resin (200-400 mesh, acetate form) which ha been previously well washed with water. The column wa eluted with 0.2 M acetic acid at a flow rate of about 0.5 ml min and 10 ml fractions collected. The eluate was continuous monitored at 280 nm by means of an L.K.B. Uvicord system and radioactivity in the fractions was detected by countir 1 ml of the samples mixed with 10 ml of scintillator (toluen 1 1; Triton X 100, 500 ml; 2,5-diphenyloxazole, 5 g) in Packard Tri-Carb liquid scintillation spectrometer.

The dopamine 3-O-35S-sulphate was eluted between 900 and 1,250 ml and the 4-O-35S-sulphate between 1,300 and 1,700 m

<sup>&</sup>lt;sup>1</sup> Lin, J. Y., Jeng, T. W., Chen, C. C., Shi, G. Y., and Tung, T. C., Nature, 246, 524 (1973).

Table 1 Daily excretion of dopamine sulphoconjugates by Parkinson patients on L-dopa therapy							
G. I. i.		Dose of	Dopamine	Dopamine	Total	Dopamine	Dose excreted
Subject		L-dopa (g per 24 h)	3-O-sulphate (mg per 24 h)	4-O-sulphate (mg per 24 h)	dopamine sulphoconjugate	3-O-sulphate (% of total)	as dopamine sulphoconjugate
		(D I	(	( 0)	(mg per 24 h)	(70)	(%) 7.8
1 male, 54 yr		4	292.9	78.2	371.1	78.9	7.8
2 male, 38 yr		3	245.3	39.5	284.8	86.1	8.0
3 male, 70 yr		3	97.5	16.2	113.7	85.7	3.2
4 male 52 vr	•	2	242 1	45 3	287 4	84.2	12.2

(as determined in preliminary experiments). Two peaks of ultraviolet absorption which were clearly separated from the main absorbing band and exactly coincident with the two peaks of radioactivity were observed (Fig. 1). The fractions from the column representing each sulphoconjugate were pooled and the radioactivity of each was measured on 1 ml aliquots to determine ■the total recovery of the sulphoconjugates from the column. A portion (100 ml) of each sulphoconjugate fraction was vophilised and a sample (1 ml) of the solution obtained by -dissolving the residue in 4 ml of water was subjected to ■hydrolysis by heating at 100° C with 2 ml of 0.3 M HCl for 40 min. This procedure had been shown in previous experiments ◆o hydrolyse 96.0% of both the 3-O and 4-O-sulphates without destruction of the liberated dopamine which could then be -determined. Portions (2 ml) of the hydrolysates were then -diluted with water to 250 ml for the 3-O-sulphate and to 25 ml for the 4-O-sulphate fractions. This dilution of the fractions was sufficient to make adjustment of their pH unnecessary. The diluted hydrolysates were then analysed for dopamine ■using the trihydroxyindole procedure. The purity of the hydrolysates with respect to dopamine was confirmed by an examination of their excitation and emission spectra. The presence of other catecholamine derivatives could not be

The amount of dopamine sulphoconjugate excreted by these Parkinson patients is comparable with that found by other workers although this was not the main concern of the present study. The results however show that the 3-O-sulphate is certainly the predominant conjugate and is consistently so, even in patient no. 3 where the total amount excreted is rather •ow. It therefore seems that the sulphoconjugation of dopamine n the human follows a pattern similar to that in the rat and an result in the rapid removal of an appreciable amount of

detected. The results were corrected for the amount of 35S-

95%) from the ion-exchange columns and also the efficiency

■abelled sulphoconjugate added at the beginning, the recovery

of the hydrolysis procedure (Table 1).

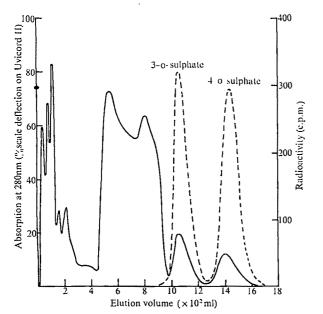


Fig. 1 Elution profile of urinary dopamine sulphoconjugates. Absorption at 280 nm; - - - , radioactivity.

administered dopa from the system. It is also noticeable that the amount of amine sulphoconjugated becomes considerable when dopa is administered orally, emphasising the possible role of the digestive tract in this process. Attention has been drawn to this point in earlier studies on the metabolism of related compounds8. In the mammalian system O-sulphation and O-methylation appear to occur predominantly on the same OH group and they may therefore represent competing processes. The predominant position of O-methylation in various catechols is not only dependent upon the relative nucleophilicities of the phenolic groups but is also strongly influenced by the polarity and orientation of the substituent side chain and other factors. In the case of dopamine, 3-O methylation is favoured near physiological pH. Although less is known about the effect of such factors on the O-sulphation of catechol derivatives, it seems that the situation is somewhat similar (unpublished results). The site of formation of excreted dopamine sulphoconjugates in the system now needs to be established. The identity of the dopamine sulphoconjugate to be found in the tissues<sup>2</sup> and in the blood where it has been claimed to be the only detectable form of the amine<sup>10</sup>, should also be investigated.

The method developed for the identification of the metabolites seems to be generally applicable (W.N.J. and F.A.R., unpublished) and can be of use for the analysis of normal human urine where the excretion pattern is similar although the concentrations are appreciably lower<sup>11</sup>.

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# Displacement of bound <sup>14</sup>C-fluphenazine by biogenic amines and antipsychotic drugs in homogenates of brain tissue

Considerable evidence suggests that antipsychotic drugs block dopamine receptors at postsynaptic sites in the central nervous system1,2. Receptor sites for neurotransmitter substances, such as acetylcholine<sup>3-5</sup> and glycine<sup>6</sup>, have been studied by measurements of the direct binding of radiolabelled compounds that have high affinity for such sites and cause receptor blockade. In a similar study of biogenic amine receptor sites, I have measured the binding and displacement of the potent phenothiazine antipsychotic drug, fluphenazine, to preparations of brain tissue *in vitro*. I have found that fluphenazine binds to all regions of the rat brain, and that the potency of antipsychotic drugs in displacing fluphenazine correlates well with their clinical efficacy. Dopamine does displace fluphenazine from binding sites, but this is a nonspecific effect shared by other biogenic amines.

<sup>14</sup>C-fluphenazine (4-[3-<sup>14</sup>C-3-(2-trifluoromethyl) phenothiazin-10-yl) propyl]-1-piperazine ethanol, dihydrochloride, 2.4 mCi mol<sup>-1</sup>) was synthesised by F. Dondzila (Chemical Development Department, Squibb Institute) by first reacting 1-chloropropyl-3-<sup>14</sup>C-bromide (3 mCi mmol<sup>-1</sup>, New England Nuclear Corp.) with 2-(trifluoromethyl) phenothiazine to form 10(1-<sup>14</sup>C-3-chlorpropyl)-2-(trifluoromethyl) phenothiazine. This compound was refluxed in the presence of 2-piperazine ethanol to form <sup>14</sup>C-fluphenazine base from which the dihydrochloride salt was obtained. <sup>14</sup>C-fluphenazine dihydrochloride was recrystallised twice from absolute ethanol to yield 98.5% radiochemical purity, which was determined by paper chromatography in two solvent systems, CHCl<sub>3</sub>:ethanol:ammonia, 80:10:1 and CHCl<sub>3</sub>:95% ethanol:ammonia, 10:85:25.

Male Sprague-Dawley rats (180-220 g) were killed by cervical dislocation and decapitated, and their brains were removed immediately. Samples of brain tissue were homogenised in 100 volumes of 0.05 M sodium potassium phosphate buffer (pH 7.4) containing 0.01% Triton X-100 using a Polytron ultraspeed homogeniser. In the standard binding assay, aliquots (10-100 µl) of a striatal homogenate (0.01-0.1 mg protein) were added to 2 ml of phosphate buffer containing 1 µM <sup>14</sup>C-fluphenazine (13,500 c.p.m.) alone or in the presence of 100 mM dopamine or 0.1 mM unlabelled fluphenazine. The mixture, in a 20-ml glass beaker, was incubated at 37° C for 10 min and then poured into a polypropylene centrifuge tube. The beaker was then washed with 1 ml of phosphate buffer. After centrifugation at 40,000g for 10 min, the supernatant fluid was decanted and the pellet was rinsed with 10 ml of phosphate buffer. Bound radioactivity was extracted into 1 ml of ethanol and 10 ml of dioxane-naphthalene liquid scintillation fluid and assayed using a Beckman LS 355 liquid scintillation counter. Counting efficiency for 14C was

Displaceable <sup>14</sup>C-fluphenazine-binding was obtained by subtracting from the total bound radioactivity the amount not displaced by high concentrations of dopamine (100 mM) or fluphenazine (0.1 mM). In a typical assay measuring the binding of <sup>14</sup>C-fluphenazine to a sample of striatal homogenate (0.05 mg protein), 2,400 c.p.m. of <sup>14</sup>C-fluphenazine was bound, of which 70% was displaced in the presence of excess dopamine or unlabelled fluphenazine. The same maximal displacement of bound <sup>14</sup>C-fluphenazine was obtained with excess nonradioactive fluphenazine or dopamine.

Displaceable <sup>14</sup>C-fluphenazine-binding was linear between 0.01 and 0.1 mg of striatal protein (Fig. 1) and was saturable with increasing concentrations of <sup>14</sup>C-fluphenazine. Half-maximal binding of <sup>14</sup>C-fluphenazine to samples of striatal homogenate (0.05 mg protein) occurred at 4.9 µM unlabelled fluphenazine (Fig. 1), as compared with half-maximal displacement by unlabelled fluphenazine, which occurred at 4.6 µM. As half-maximal saturation occurs at nearly the same concentrations of unlabelled and <sup>14</sup>C-fluphenazine, this indicates that <sup>14</sup>C-fluphenazine is biologically equivalent to the nonradioactive drug in terms of tissue binding. The binding of <sup>14</sup>C-fluphenazine in the presence of excess dopamine or nonradioactive fluphenazine was not saturable and increased linearly with increasing <sup>14</sup>C-fluphenazine or tissue concentration.

The optimal pH for displaceable <sup>14</sup>C-fluphenazine binding was 7.4, with a steep decline in binding at more acid and alkaline

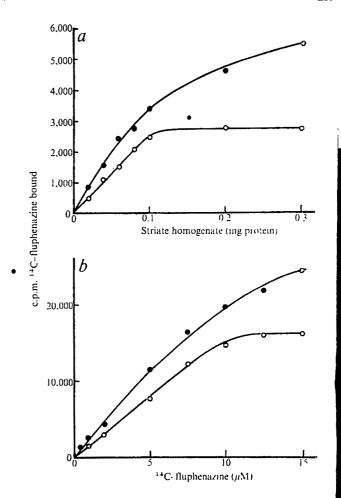


Fig. 1 a, Tissue linearity of displaceable <sup>14</sup>C-fluphenazine binding to components of homogenates of the striate region of rat brain. Dilutions of a 1 in 100 homogenate of striatal tissue incubated with 1 μM <sup>14</sup>C-fluphenazine alone or in the presence of 100 mM dopamine in the standard binding assay. Displaceable <sup>14</sup>C-fluphenazine binding was obtained by subtracting from the total bound radioactivity the radioactivity bound in the presence of dopamine. Each value is the mean of triplicate determinations, with variations of less than 10%. b, Binding of <sup>14</sup>C-fluphenazine to striatal homogenates as a function of the concentration of fluphenazine. The binding of various concentrations of <sup>14</sup>C-fluphenazine alone or in the presence of 1 mM nonradioactive fluphenazine to samples of striatal homogenate (0.05 mg protein) were measured using standard assay procedure. Displaceable <sup>14</sup>C-fluphenazine binding was estimated by subtracting from the total bound radioactivity the radioactivity bound in the presence of excess unlabelled fluphenazine. Each value is the mean of triplicate determinations, which differed from one another by less than 10%. •, Total, (), displaceable.

values. Displaceable binding occurred at incubation temperatures from 4 to 40° C, with a rapid decline at higher temperatures. If, however, striatal homogenates were subjected t 100° C for 10 min, cooled and then incubated with <sup>14</sup>C-flupher azine at 37° C, the displaceable fluphenazine-binding wareduced by only 30%. This suggests that the temperature dependence of the incubation step is a result of the instability of fluphenazine and catecholamines at higher temperature rather than destruction of binding sites. Nondisplaceable <sup>14</sup>C-fluphenazine-binding was not dependent on either pH of temperature. High concentrations (5–100 mM) of Na+, K-Li+, Ca<sup>2+</sup> or Mg<sup>2+</sup> in the incubation medium did not affed displaceable fluphenazine-binding.

The association and dissociation of displaceable <sup>14</sup>C-fluphe azine binding to striatal homogenates as measured by the ra of displacement by 1 mM unlabelled fluphenazine was rapibeing fully equilibrated within less than 2 min. Reliable d terminations of binding at intervals less than 1 min require

Table 1 Subcellular distribution of <sup>14</sup>C-fluphenazine binding in the striate region of rat brain

<sup>14</sup> C-fluphenazine bound (c.p.m. per mg protein)		
Total	Displaceable	
37,960	27,640	
45,020	29,740	
60,360	41,720	
55,400	37,460	
ns		
25,620	13,260	
64,380	41,740	
	per mg Total 37,960 45,020 60,360 55,400 ns	

Tissues were prepared and subjected to differential centrifugation '\ sample of P<sub>2</sub> pellet was resuspended in 20 volumes of ice-cold listilled water and homogenised with a ground glass homogeniser and centrifuged for 20 min at 9,000g. The supernatant fluid was collected and the pellet, a bilayer with a soft buffy upper coat, was insed with the supernatant fluid to collect the upper layer. The supernatant fluid was then centrifuged at 48,000g for 20 min. The sellets obtained were suspended in 0.5 M sodium potassium phosphate suffer (pH 7.4) containing 0.01% Triton X-100. The data shown represent the mean of four experiments in which the variations among the results were less than 15%. Protein was determined by the nethod of Lowry et al.9 with bovine serum albumin as the reference standard.

nore rapid separation technique, such as filtration, in order
o separate tissue from the incubation media more quickly;
he high degree of binding of <sup>14</sup>C-fluphenazine to various filter
 naterials prevented the use of such a technique.

Endogenous levels of dopamine are highest in the striate egion of the brain, in which most of this amine is localised → dopaminergic nerve terminals<sup>7</sup>; therefore, if displaceable <sup>4</sup>C-fluphenazine binding is specific for dopamine receptors, egional studies of the brain should indicate more binding -o homogenates of the striate region than to other portions of he brain. Moreover, subcellular studies may be expected to -ndicate that the greatest binding to the crude mitochondrial -raction, which contains 'pinched off' nerve endings and the ssociated postsynaptic membrane, in which the neurotransnitter receptor sites are thought to be localised. The crude -aitochondrial fraction prepared from the striate region of he brain exhibts most displaceable 14C-fluphenazine binding, aut other subcellular fractions, in particular the crude microomal pellet, also have extensive binding capacity (Table 1). fter the crude mitochondrial pellet was subjected to hypo-osmo-

able 2 Distribution of <sup>14</sup>C-fluphenazine-binding to brain regions and selected peripheral tissues of the rat

<sup>14</sup> C-flup	henazine bound (c.p. Total	.m. per mg protein) Displaceable
pinal cord	$39,800 \pm 2,980$	$21,500 \pm 1,990$
1edulla oblongata-pons	$44,400 \pm 3,240$	$28,860 \pm 2,240$
'erebellum	$43,400 \pm 4,010$	$28,560 \pm 2,640$
fidbrain	$40,200 \pm 3,860$	$24,880 \pm 2,280$
*(ypothalamus	$40,000 \pm 3,940$	$24,280 \pm 2,140$
halamus	$34,700 \pm 3,260$	$20,020 \pm 1,960$
≪ippocampus	$40,200 \pm 2,760$	$24,400 \pm 1,840$
orpus striatum	$44.200 \pm 2.870$	$30.580 \pm 2.160$
erebral cortex	$48,200 \pm 3,160$	
elebrar cortex	46,200 ± 3,100	$30,980 \pm 2,450$
eart-atrium	$10,680 \pm 1,180$	$4,400 \pm 320$
eart-ventricle	$23,400 \pm 1,960$	$10.960 \pm 870$
orta	$22,700 \pm 2,080$	3.340 + 310
iver	$13,750 \pm 1,160$	$980 \pm 110$
<ung< td=""><td><math>9,300 \pm 840</math></td><td><math>1,860 \pm 160</math></td></ung<>	$9,300 \pm 840$	$1,860 \pm 160$
idney	$23,300 \pm 2,210$	$9.180 \pm 460$
mall intestine	$13,500 \pm 1,310$	$6,800 \pm 540$
riated muscle	$3,710 \pm 380$	680 + 80
	-,	222 1

The data shown represent the mean  $\pm$  s.e.m. of triplicate determinations of two experiments.

tic shock, the displaceable 14C-fluphenazine binding was restricted primarily to the subfraction containing synaptic membranes. This suggests that the displaceable 14C-fluphenazine-binding sites are consistent with postsynaptic dopamine receptors in the striate region; however, regional studies of the brain revealed a rather uniform pattern of displaceable <sup>14</sup>C-fluphenazine binding (Table 2). Although most binding occurred in the striate region and the cerebral cortex, there was no correlation between the regional concentration of dopamine and the displaceable 14C-fluphenazine binding capacity. The displaceable binding to peripheral organs and tissues was much less than that observed in brain tissue, with the liver and a sample of striated muscle having the lowest binding capacity of the tissues studied. The regional and subcellular distributions of displaceable fluphenazine binding were similar when the concentration of <sup>14</sup>C-fluphenazine in the incubation medium was reduced to 0.5 µM, the limit of sensitivity of the assay system.

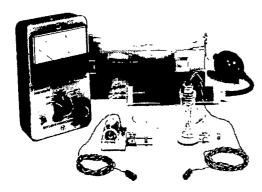
Table 3 Relative potencies of drugs in reducing displaceable <sup>14</sup>C-fluphenazine binding to homogenates of the striate region of rat brain

Drug ·	$ED_{50}(\mu M)$	No effect	(mM)
Fluphenazine		Acetylcholine	100
Thioridazine	8.4	GABA	100
Chlorpromazine	32	Glutamic acid	100
Haloperidol	35	Glycine	100
Spiroperidol	41	Pargyline	1
Clozapine	62	Iproniazid	1
Pimozide	380	Pentobarbitone	10
Promethazine	430	Thiopentone	10
Imipramine	450	Diazepam	10
Benztropine	460	Chlordiazepoxide	10
Propranolol	500	Dopa	100
Dichlorisoprenaline	600	Homovanillic acid	100
Tranylcypromine	900	3-Methoxy-4-hydroxyphenyl	
•		glycol	100
Dexchlorpheniramine	1,000	Adrenochrome	100
Phentolamine	1,200	Atropine	1
Phenoxybenzamine	1,900		
Methysergide	4,800		
d-Amphetamine	7,500		
Clonidine	17,000		
<i>l</i> -Noradrenaline	28,000		
Apomorphine	29,000		
Dopamine	30,000		
6-Hydroxydopamine	34,000		
I-Adrenaline	35,000		
Histamine	40,000		
Tyramine	42,000		
Serotonin	42,000		
Isoprenaline	44,000		
Normetanephrine	51,000		
d-Adrenaline	55,000		
Metanephrine	61,000		

The values shown represent the mean of two or three log-probit determinations using four concentrations of the drug.

In most regions of the brain the predominant biogenic amines are noradrenaline and serotonin and in all brain regions and subcellular fractions of the striate or cerebellum these amines could displace bound 14C-fluphenazine with potencies very similar to that of dopamine (Table 3). Other putative neurotransmitters, such as acetylcholine,  $\gamma$ -aminobutyric acid (GABA), glutamic acid and glycine, were inactive in this regard. The displacement of  $^{14}$ C-fluphenazine from binding sites by catecholamines, serotonin, their metabolites and related amines does not necessarily mean that these amines will have agonist activity at such sites; they may be very weak antagonists. This hypothesis is supported by the finding that various drugs, including α and β-receptor blockers and tricyclic antidepressant compounds, displace bound 14C-fluphenazine (Table 3); in this respect they are not as potent as compounds with known antipsychotic activity. The phenothiazine antipsychotics fluphenazine, thiorodazine and chlorpromazine were the most potent in displacing bound <sup>14</sup>C-fluphenazine, whereas pro-

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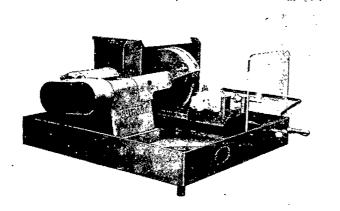
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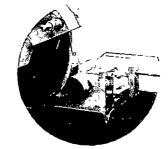
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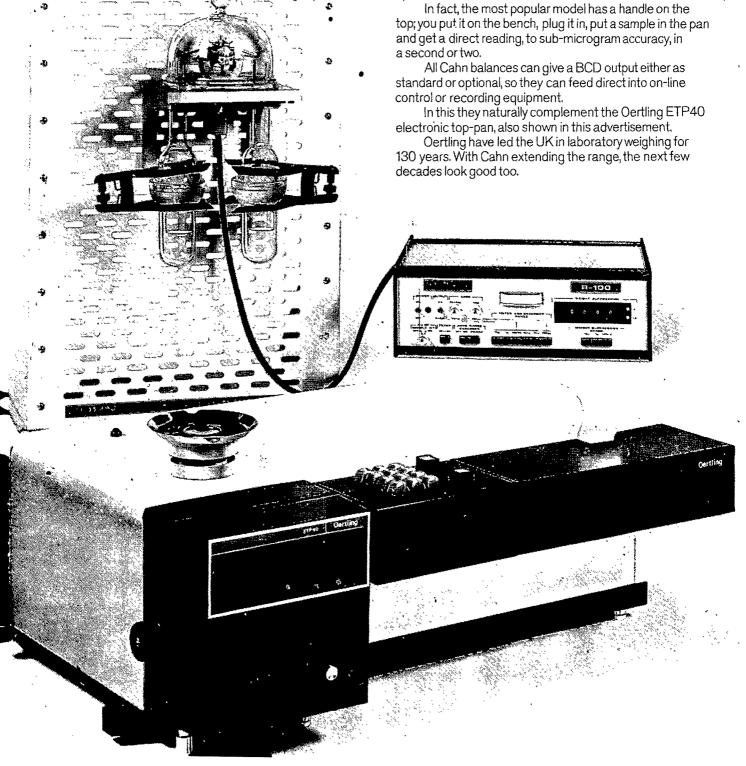


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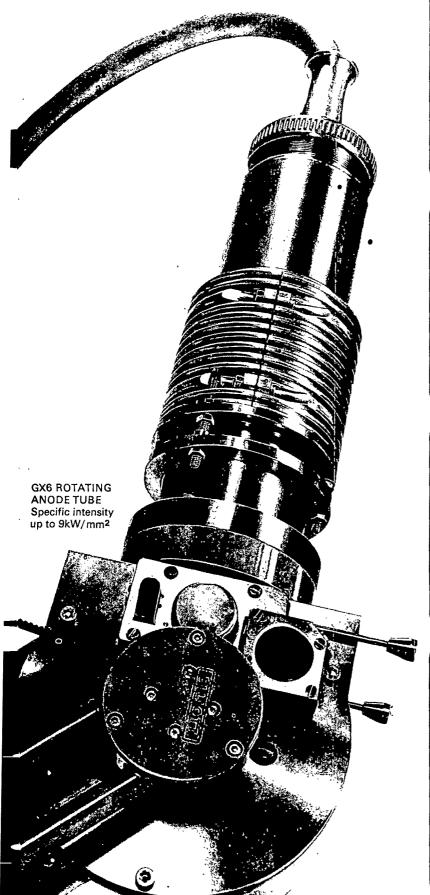
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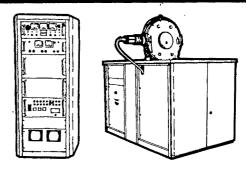
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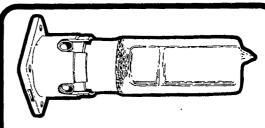
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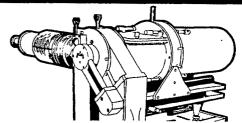
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Elstree Way, Borehamwood, Herts., England. Tel: 01-953 2030 - Telegrams: Elliotauto Borehamwood Telex: 22777 Member of GEC-Marconi Electronics Ltd methazine, a phenothiazine with weak antipsychotic properties, was about 100 times less active than fluphenazine. All compounds tested that possessed antipsychotic activity could displace bound 14C-fluphenazine in the striate region and cerebellum and there was a potency correlation between these two effects. A similar correlation exists between the clinical efficacy of anitpsychotic drugs and their ability to block the activation of adenyl cyclase by dopamine in selected brain regions<sup>1,2</sup>. Although dopamine did have affinity for fluphenazine binding sites, the variety of biogenic amines and related compounds that could displace bound fluphenazine in all brain regions suggested that dopamine receptors were not specifically labelled at the concentration of fluphenazine used, which is higher than that required to block the dopamine receptor of adenyl cyclase<sup>1</sup>. Therefore, although my studies support indirect evidence that antipsychotic drugs may act by blockade of receptor sites for biogenic amines, they also suggest that this action is not confined to striatal dopamine receptors at postsynaptic sites.

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#### Tyrosinase in the skin of albino hamsters and mice

Measurements of tyrosinase (EC 1.10.3.1) in animals with oculocutaneous albinism have led to contradictory conclusions. Using the incorporation of <sup>14</sup>C-DL- or L-tyrosine into melanin, one investigator found no, or questionable, activity in albino mouse skin1,2 while another3 reported very low, but significant activity in particles from albino mouse eyes treated with Triton X-100. When the production of <sup>3</sup>HOH from 3,5-<sup>3</sup>H-L-tyrosine was used as an assay, albino hamsters' were found to have no detectable activity, while albino rats' were reported to have minimal, but real activity. We report clear evidence for a tyrosine hydroxylase with tyrosinase characteristics in the skin of two strains of albino hamster and one strain of albino mouse. The method used permits quantitative comparison with tyrosinase in pigmented skin. A distinct dopa oxidase is present in extracts of albino mouse.

Tyrosine hydroxylating activity of tyrosinase was measured<sup>4</sup> by incubating (at 37° C) 3,5-3H-L-tyrosine, 1 μmol (2 to 3×10<sup>6</sup> d.p.m.); L-3,4-dihydroxyphenylalanine (dopa), 0.15 µmol; sodium phosphate (pH 6.8) or sodium pyrophosphate (pH 7.4) 20-30  $\mu$ mol; and enzyme in a total volume of 1.25 ml. Where indicated in the Tables dopa was omitted. When diethyldithiocarbamate (DDC) was added the components were incubated together for 1 h at 37° C before addition of tyrosine and dopa. The blanks were reactions without enzyme; blanks have been subtracted from observed values to obtain the values reported (averages

Table 1 Tyrosine hydroxylation by skin homogenates from T<sup>5</sup> albino and Syrian golden hamsters

Experiment and	Dopa	DDC	3HOH
type of hamster	$(1.2 \times 10^{-4} \text{ M})$	$(1\times10^{-3} \mathrm{M})$	(nmol h -1 g -1 sk
ι	+	_	23
(1) TWA albino		_	21
(-)	+	+	23
	*	• 🗼	26
(2) Syrian golden	+	<u>-</u>	372
	*****	_	30
	+	+	40

In both experiments skin from 7-d-old hamsters was homogeni in 0.02 M sodium pyrophosphate (pH 7.4) (1 g skin per 3 ml). 7 standard assay mixture for tyrosine hydroxylation was used exce where indicated, dopa was absent and DDC was added. The blas were equivalent to 9.0 nmol h<sup>-1</sup> g<sup>-1</sup> (experiment 1) and 60 nmol h<sup>-1</sup> (experiment 2).

of duplicates). 3,5-3H-L-tyrosine was purified by high volta paper electrophoresis6 and 3HOH in the stored 3H-tyrosi was removed by evaporating the solution to dryness imme iately before use. Boiled homogenates gave the same resu as non-enzymatic controls.

Another assay for tyrosinase was based on the oxic tion of dopa to dopachrome observed spectrophotometrica at 475 nm (ref. 7). A unit of tyrosinase is the amor catalysing the formation of 1 µmol of dopachrome min

Two strains of albino hamsters were used (Lakevi Hamster Colony, Newfield, New Jersey). TWA is oculocutaneous albino with no black areas while MI is similar but the adults develop pigmented skin in f ears. AKR/J albino mice were obtained from the Jacks Laboratory. The animals were killed between ages 5 and 7 either by decapitation or quick freezing. The body sk were frozen until used.

Antiserum against homogeneous hamster melanoma ty sinase8 was raised in rabbits. The antiserum was titred follows. Antiserum (0.10 ml, diluted with 10% norn rabbit serum (NRS) made up in 0.05 M sodium bors (pH 8.5)) was incubated at 37° C for 1 h with tyrosins (0.02-0.2 U) and sodium phosphate (pH 6.8), 30-60  $\mu$ m in a volume of 1 or 2 ml. The solution was then centrifug at 105,000g for 1 h and a portion of the supernatant vassayed for tyrosinase by either the 3HOH or the do; chrome method. The enzyme remaining in the supernata

Table 2 Dopa dependency and DDC inhibition of tyrosine hydro lation by supernatant fractions of skin from TWA and MHA albi hamsters

Experiment and type of hamster	Dopa (1.2×10 <sup>-4</sup> M)	DDC (1×10 <sup>-3</sup> M)	Time of incubation (h)	³HOH (nmol g
•	+		6	8.3
(1) TWA albino		_	6	0.8
•	+	+	6	. 0
(2) TWA albino	+	_	6	13.3
• •	+	+	6	0
	+	_	3	9.2
(3) MHA albino		_	3	2.5
, ,	+	+	3	0
(4) MHA albino	+	_	6,5	11.7
•	·	_	6.5	6.3

Homogenates of albino hamster skin were prepared as described Table 1. After centrifugation at 105,000g for 1 h the supernata were dialysed against 0.003 M sodium phosphate (pH 6.8) and stirt with calcium phosphate gel (1.75 mg gel per mg protein) for 1 h. mixtures were centrifuged at 10,000 r.p.m. for 20 min and the sup natants were assayed. The standard assay procedure for tyros hydroxylation was used, with dopa absent and DDC included what appropriate. Blanks were equivalent to 5.8 nmol g<sup>-1</sup> (experiment 4.0 nmol g<sup>-1</sup> (experiment 2), 7.5 nmol g<sup>-1</sup> (experiment 3), a 4.6 nmol g<sup>-1</sup> (experiment 4). was always compared with a control incubated only with NRS. No loss of activity was seen in control samples.

The tyrosine hydroxylating activity of TWA hamster albino skin (Table 1) was at a level which was missed previously. Table 1 also shows that hydroxylation by TWA homogenates was not dependent on dopa and not inhibited by DDC as was tyrosinase in homogenates of pigmented hamster skin. Similar results were obtained from homogenates of MHA albino skin. Centrifugation of his homogenate at 105,000g for 1 h gave a supernatant which had 10–15% of the tyrosine hydroxylation activity. Treatment of these supernatant fractions with calcium phosphate gel gave fractions which were dependent on dopa and inhibited by DDC, properties typical of tyrosinases (Table 2).

The AKR/J albino mouse skin homogenate had a tyrosine hydroxylating activity of 0.2  $\mu$ mol h<sup>-1</sup> g<sup>-1</sup>, independent of dopa and not inhibited by DDC. Although little or no activity was released by homogenisation in buffer alone, 10–25% of the activity was extracted with Igepal CO-630 (nonylphenoxypoly (ethyleneoxy) ethanol; GAF, New York.) The data in Table 3 show that the activity in the calcium phosphate fraction was inhibited by DDC and that in the ammonium sulphate fraction was dependent on dopa.

An immunological demonstration that tyrosine hydroxylation belonged to a tyrosinase-like protein was based on the use of antibody against tyrosinase. This is clearly shown in Table 4. The activity in skin from each of the three albino strains was bound to antibody and sedimented during centrifugation. This led to the loss of tyrosine hydroxylation activity from the supernatant.

Tyrosinase possesses both tyrosine hydroxylating and dopa oxidising activities, the latter property leading to the formation of melanin. Surprisingly, it was possible to separate a dopa oxidase from the tyrosine hydroxylase as follows. Supernatant from a AKR/J homogenate, 10 ml (equivalent to 3.3 g skin), prepared with Igepal, was loaded on a 2×5 cm column of DEAE-cellulose or CM-cellulose. The resin was washed with 0.003 M sodium phosphate **◄**(pH 6.8), 150 ml (fraction 1, containing most of the Igepal) and 0.1 M sodium phosphate (pH 6.8), 160 ml (fraction 2). The fractions were concentrated under N2 about 15-fold with an Amicon PM-10 filter. Qualitatively the results from both resins were the same. Fraction 1 from each column retained the tyrosine hydroxylating activity, while fraction 2 had none. While fractions 1 formed some pigment, however, the fractions 2 became quite dark during incubation, indicating that these fractions retained most of the dopa oxidase activity. In one experiment, 0.8 ml of fraction 2

Cable 3 Dopa dependency and DDC inhibition of tyrosine hydroxylaion by supernatant fractions of skin from AKR/J albino mice

Experiment	Dopa	DDC	³НОН
	$(1.2 \times 10^{-4} \text{ M})$	$(1 \times 10^{-3} \text{ M})$	$(nmol g^{-1})$
1) Calcium	` + ´	` _ ′	180
phosphate gel	+	+	5.0
fraction		<u>-</u>	162
2) Ammonium	+	_	44.4
sulphate fraction		-	13.8

AKR/J albino mouse skin was homogenised as in Table 1 in buffer ontaining 1% by volume of Igepal. After centrifugation at 105,000g or 1 h, the supernatant was treated with calcium phosphate gel as tescribed in Table 2. The supernatant was then treated with amaonium sulphate to 80% saturation. On centrifugation of this nixture the protein precipitate remained suspended with Igepal at the op of the tube. This protein-Igepal mixture was separated and disolved in 0.05 M sodium phosphate (pH 6.8) to give a milky suspension which was dialysed against the same buffer. In experiment 1 the alcium phosphate gel fraction was used; in experiment 2, the immonium sulphate fraction. The standard assay for tyrosine sydroxylation (6 h incubation) was used with dopa absent and DDC colleded where appropriate. Blanks were equivalent to 15 nmol g<sup>-1</sup> (experiment 1) and 3.5 nmol g<sup>-1</sup> (experiment 2).

Table 4 Binding of tyrosine hydroxylating activity in extracts of TWA and MHA albino hamster and AKR/J albino mouse skin to anti-tyrosinase serum

Experi- ment	Extract source	Equivaler amount of		NRS	³HOH in aliquot	Activity lost from
(1)	TWA ·	skin (g)			counted (d.p.m.) 476	superatant (%)
(1)		4.0	+	+ - +	197 1,732	58
(2)	AKR/J	0.8	+	<del>-</del>	1,732 0 483	100
(2)	Hamster (		+	· <del>-</del>	157 9,480	77
	melanoma		+	_	4,675	51

Calcium phosphate gel extracts of skin from TWA albino hamsters (experiment 1) and MHA albino hamsters, AKR/J albino mice, and hamster melanoma\* (experiment 2) were incubated with antiserum to hamster melanoma tyrosinase (final concentration 1:3,500) or an equivalent amount of NRS. The solution contained 2 mg (experiment 1) or 4 mg (experiment 2) bovine serum albumin, sodium phosphate buffer (pH 6.8), 60 µmol, in a final volume of 1.14 ml (experiment 1) or 1.24 ml (experiment 2). The incubation was for 1 h at 37° C followed by 16 h at 4° C and then centrifugation at 100,000g for 1 h. Supernatant (0.5 to 0.8 ml) was assayed for residual tyrosine hydroxylating activity by the standard method. Time of incubation was 6 for the albino skin extracts and 3 h for the melanoma tyrosinase. Blank values were 400 d.p.m. (experiment 1) and 555 d.p.m. (experiment 2).

oxidised 1.8 nmol dopa min<sup>-1</sup> but the fraction had no tyrosine hydroxylating activity. Hamster melanoma tyrosinase, possessing the same dopa oxidase activity, hydroxylated 16 nmol tyrosine h<sup>-1</sup>.

An inhibitor of the dopa oxidase reaction of tyrosinase was present in fraction 1—0.40 ml completely inhibited dopa oxidation by melanoma tyrosinase.

Skin from newborns of all three albino animals can hydroxylate tyrosine at a rate 2-3% that for the corresponding pigmented variety (Syrian golden hamster4 and C57BL/6J mouse<sup>9</sup>). The amount solubilised by homogenisation alone (hamster) or by Igepal treatment (mouse) amounts to about 10% of that assayed in the homogenate. Further purification gives fractions which are dopa-dependent, inhibited by DDC and bound by anti-tyrosinase serum. Whether all the activity in the homogenates relates to tyrosinase cannot be decided yet. It seems unlikely that any of it is the result of a tetrahydropteridine-dependent tyrosine hydroxylase because even the crude adrenal medullary enzyme10 exhibits an absolute requirement for a pteridine cofactor. The absence of dopa-dependency could be explained by the presence of elevated levels of dopa or some other nonspecific reducing agent. The lack of inhibition by DDC cannot be readily explained. One investigator<sup>3</sup> inhibited albino eye tyrosinase with  $1 \times 10^{-2}$  M DDC, while another<sup>5</sup> reported success with albino skin using 2×10<sup>-4</sup> M DDC.

Triton treatment did not further stimulate the activity probably because freeze—thawing had already disrupted melanosomal membranes<sup>11</sup>. We were, however, also unable to confirm with skin, Hearing's observation<sup>3</sup> that albino eye homogenates are stimulated by Triton to a level equal to that of pigmented eye.

A distinct dopa oxidase is present in albino mouse skin. The tyrosine hydroxylation fraction still retains some dopa oxidase activity, but the presence of a dopa oxidase inhibitor in that fraction complicates the picture. It is uncertain how these findings relate to human albinism. Hair bulbs from one type of human albino<sup>12</sup> develop slight pigmentation when they are incubated for long periods with tyrosine.

Inhibitors of tyrosinase have been reported in albino eye<sup>3</sup> and many other tissues<sup>13</sup>. Their physiological role, however, remains obscure.

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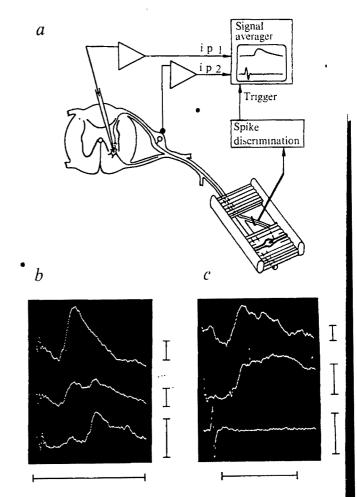
#### Monosynaptic excitation of motoneurones from secondary endings of muscle spindles

SINCE Lloyd's original work on the myotatic unit more than 30 yr ago<sup>1</sup>, it has been assumed that the monosynaptic excitation of motoneurones from receptor nerve fibres derives entirely from the Group Ia afferents, which terminate as primary endings in the muscle spindles. Group Ib afferents from tendon organs are thought to exert their inhibitory actions<sup>2</sup> over di- or trisynaptic pathways3, while Group II and III fibres, which include those from the secondary endings of muscle spindles, form part of the flexor reflex afferent (FRA) system4, the synaptic actions of which are thought also to be mediated polysynaptically5. Whereas the monosynaptic excitation of motoneurones by Group Ia fibres has been directly confirmed in terms of unitary synaptic potentials evoked by single, stretch excited primary endings6, knowledge of the synaptic actions of fibres innervating secondary endings is known only indirectly from electrical stimulation of Group II fibres and is not without controversy7.

Here, avoiding the use of electrical stimulation, we have investigated the synaptic actions exerted on thoracic motoneurones by afferent fibres from primary and secondary endings of intercostal muscle spindles excited by stretch. We show that contrary to all previous beliefs, monosynaptic excitation of motoneurones can derive from secondary as well as primary

Cats anaesthetised with sodium pentobarbitone, paralysed with gallamine triethiodide and artificially ventilated were prepared for intracellular recording from expiratory motoneurones of the thoracic spinal cord at T8 as previously described8. One or several intramuscular nerve filaments supplying the internal intercostal muscle of T8 were freed but left in continuity9. Afferent impulses recorded from them were used to trigger a signal averager thus extracting from the intracellularly recorded synaptic noise averaged synaptic potentials evoked by individual receptor nerve fibres6

All signals were recorded on magnetic tape, which enabled subsequent detailed analysis of the synaptic potentials evoked by a large number of afferent fibres using the method shown



a, Experimental set-up. b, Spike-triggered average responses in an expiratory motoneurone. Three different window' settings of the spike discriminator, using as triggers from above down, large, medium and small amplitude spikes. Upper two traces, averages of 8,192 sweeps; lowest trace, average of 16,384 sweeps. Conduction distance, 47 mm to the ventral horn. c, Averaged responses evoked in two different expiratory motoneurones by the same, single unit trigger spike, together with its action potential averaged from the surface of the dorsal root ganglion (lowest trace). This unit showed a low dynamic sensitivity and a very regular firing pattern. Upper trace, average of 1,024 sweeps; lower two traces, averages of 8,192 sweeps. Conduction distances: 29 mm to the ganglion and 43 mm to the ventral horn. Calculated conduction velocity of the afferent,  $38 \text{ ms}^{-1}$ . Calibrations, 5 ms and 10  $\mu$ V, except c, 1  $\mu$ V for lowest

schematically in Fig. 1a. The filament recording was fed to 'window' discriminator so that spikes of given amplitude range could be selected as triggers for the averager. Analysis wa carried out by repeated replaying of the tape, each time with different setting of the 'window', so as to obtain a sequence of records such as illustrated in Fig. 1b. Typically, the respons consisted of a composite synaptic potential which could b broken down into unitary synaptic potentials each defined by specific latency, having a fast rise-time (10-90% in 1.0 ms) and a maximum amplitude for a given setting of the 'window We interpret such a response as indicating that each filamen contains several receptor nerve fibres monosynapticall connected to the motoneurone concerned. This interpretation depends on the assumption that a polysynaptic pathwa would result in a synaptic potential with a much slower rise time in the averaged record because of variability in the firing times of the relevant interneurones10.

The individual latencies of the unitary synaptic potential together with an assumed value for the synaptic dela (0.5 ms) give the conduction velocities of the individual fibre concerned, and these have ranged from 24 ms -1 to 90 ms -

thus including the range given for afferent fibres from intercostal secondary as well as primary endings by Andersson, Lennerstrand and Thoden<sup>11</sup>. An error in the estimation of conduction velocity could arise, however, from slowing in the intraspinal, preterminal region of the afferent fibre 12. An independent measurement of conduction velocity was therefore obtained by recording on a separate tape channel the signal from the exposed surface of the dorsal root ganglion at T8, so that simultaneously averaged recordings were obtained of the action potentials in individual fibres at the ganglion and of the synaptic potentials they evoke (Fig. 1c). Control recordings made in a similar fashion distal to the ganglion showed that the ganglion recording gave reliable estimates of the peripheral conduction velocities. The resulting measurements gave conduction velocities which remained in agreement with both ranges of Andersson et al.11. Of 17 fibres giving monosynaptic excitation, seven had velocities above 60 ms<sup>-1</sup> and three between 60 and 40 ms<sup>-1</sup>; the remaining 6 had velocities below 40 ms<sup>-1</sup> and fell completely within the range for secondary endings given by Andersson et al. V. Individual fibres were shown to excite monosynaptically more than one motoneurone, as illustrated for the secondary ending of Fig. 1c.

All clearly distinguishable units in the filaments were sensitive to stretch and most fell into two classes. They displayed either a high dynamic sensitivity with a relatively irregular discharge or a relatively low dynamic sensitivity with a regular firing pattern, corresponding to reported properties of primary and secondary muscle spindle endings13,14. Both classes were represented among those afferents giving monosynaptic excitation. This further supports our conclusion that monosynaptic excitation of thoracic motoneurones derives from both primary and secondary muscle spindle endings.

These results have several important implications which here can be dealt with only briefly. First, motoneurones participating in the intercostal stretch reflex must draw monosynaptic excitation from secondary as well as primary endings. This stretch reflex, which has been demonstrated in cats under a variety of experimental conditions (including anaesthesia) and in conscious man, has been directly implicated in the servo -control of intercostal muscles<sup>15</sup>. Second, the relatively delayed excitation by secondary endings could provide an explanation for the long latency of the human intercostal stretch reflex, although such an explanation does not in itself -account for the absence of early servo correction by the Group a afferents<sup>16-18</sup>. Third, because of their monosynaptic connections, the secondaries, like the primaries, are favourably sited to undergo sprouting in the advent of degeneration of central nerve fibres also terminating directly on motoneurones. This postulated growth of stretch activated synapses could be the major factor in the increased resistance to stretch of spastic muscles.

Finally, we suggest that further application of these techsiques could provide direct evidence to support the hypothesis of Matthews 19,20 that secondary endings contribute excitation o the soleus stretch reflex of the decerebrate cat and thus to efute contrary evidence derived from electrical stimulation of

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Note added in proof: We have now extended these experiments to the triceps surae muscle and find the same result, that learly identified afferents from secondary endings monoynaptically excite homonymous motoneurones. This contitutes the direct evidence which we postulated above would upport Matthews' hypothesis. Our experiments so far have →een on anaesthetised preparations.

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#### Comparison of the number of anti-L-binding sites on LK goat red cells with the number of Na-K pumps

SHEEP<sup>1</sup>, goats<sup>2</sup>, possums<sup>3</sup> and some other species are unusual mammals in that the red cells of individual animals have either high (HK) or low (LK) concentrations of potassium, which correlate with the activity of the Na-K pump. The pump of LK cells is less active than that of HK cells4.5. When LK sheep red cells are injected into HK sheep an antibody of the IgG class (anti-L) is raised which, when reacted with LK sheep or goat red cells, stimulates the pump<sup>5,6</sup>. In goat cells the major, if not the only, effect of the antibody is to reduce the affinity of the pump for K at the inside cell surface, and that stimulation results from the relative increase in the effectiveness of Na as a substrate. It has been reported that in sheep, anti-L also increases the maximal pump rate<sup>8</sup>. The antibody might function by binding either to the pump, and so altering its kinetic characteristics, or to another component of the cell surface, indirectly altering the environment of the pump. If it combines with the pump, the number of anti-L molecules bound should be about the same as the number of pumps. We have now confirmed this using an estimate of the number of pumps of LK goat cells obtained with <sup>3</sup>H-ouabain<sup>9</sup>, and our own estimate of the number of <sup>131</sup>I-labelled antibody molecules bound when the pump is stimulated maximally. We used LK goat red cells as LK sheep red cells seem to contain a second antigen in addition to the antigen associated with pump stimulation5,10.

The antiserum used in these experiments was obtained from one HK sheep after immunisation with cells from a single LK sheep. No haemolysing antibodies for the red cells of the HK and LK goats used in this study were present in the serum. The antiserum was absorbed against cells from three HK sheep. The methods used for determining the number of antibody-binding sites were similar to those used in determining the number of anti-D binding sites on human red cells<sup>11</sup>. IgG was purified by

Table 1	Binding of anti-L by HK and LK goat red cells					
Antibody quantity (mg)	Molecules bound HK LK-1 LK-2				nal pump ulation LK-2	
$\begin{array}{c} 2.2  \times  10^{-3} \\ 0.7  \times  10^{-3} \end{array}$	8	41 17	42 16	1.83 1.12	1.96 1.24 .	

HK and LK goat red cells were exposed to purified antibody at 32° C for 0.5 h, the cells were separated from the antibody by centrifugation and washed in the cold, and then separated into two parts one of which was used for the determination of the number of <sup>131</sup>-labelled molecules bound and the other for the determination of the ouabain sensitive K influx in quadruplicate. It was determined that maximal stimulation was established during a 0.5-h incubation of LK cells with anti-L, and that none of the antibody was eluted from the cells during a 1-h incubation at 0° C (as judged by the pump-stimulating effect of the antibody). Fractional pump stimulation was calculated as the pump rate in the antibody-treated cells divided by the pump rate in control cells. Similar results were obtained at five other antibody concentrations.

ammonium sulphate precipitation and repeated chromatography on DEAE cellulose; immunoelectrophoresis against anti-sheep serum revealed a single band. The IgG was iodinated by the iodine monochloride method<sup>12</sup>; protein labelled with <sup>131</sup>I was diluted with IgG labelled with nonradioactive I. Labelled anti-L was absorbed on cells from one of the LK goats in the presence of a large excess of unlabelled nonimmune sheep IgG. The cells were washed and haemolysed, and the antibody was eluted from the ghosts with butanol13, the purified antibody was diluted with a large excess of unlabelled nonimmune sheep IgG and dialysed against phosphate-buffered saline. LK and HK goat cells were exposed to the purified antibody for 0.5 h at 32° C, washed, and part of the cells was then used to determine the number of 131I-labelled anti-L molecules bound and another part was used for the determination of the ouabain-sensitive K influx7. Solutions used during exposure of the cells to antibody and measurement of K influx have been described7.

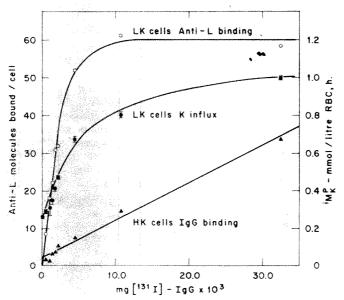


Fig. 1 The number of <sup>131</sup>I-IgG molecules bound to HK goat red cells ( $\triangle$ ) and the number of anti-L molecules bound to LK goat red cells ( $\bigcirc$ ) (calculated as the total number of <sup>131</sup>I-IgG molecules bound to the LK cells less the number bound to HK cells at the same antibody concentration) plotted against the number of mg of labelled IgG to which the cells were exposed (the total volume of solution containing the antibody was 5 ml for all points except the last, for which it was 7.5 ml). The simultaneously determined ouabain-sensitive K influx ( ${}^tM_K{}^p$ ) in the LK cells is also plotted ( $\bigcirc$ , right abscissa,  $\bot$  s.e.m., n=4) as a function of the amount of antibody to which the cells were exposed. Exposure of these cells to anti-L IgG labelled with nonradioactive I at a ratio of 1 ml of cells to 5 ml IgG (80 mg protein ml<sup>-1</sup>) resulted in a pump rate of 0.885  $\bot$  0.011 mmol per 1 red blood cells per h. The curves were drawn by eye; the HK line is a least squares plot, r=0.994.

At equal concentrations of labelled antibody, cells from the two LK animals took up about the same amount of antibody (Table 1). The number of labelled molecules taken up per cell by the HK cells was a significant percentage of the number taken up by the LK cells; this uptake was thought to represent nonspecific absorption of labelled antibody14. Since the total number of antigen sites is so low on LK cells, the elution process (which presumably removes both specifically bound and nonspecifically bound IgG) does not result in a monospecific antibody preparation. In calculating the number of anti-L molecules bound to LK cells, the number of molecules bound to HK cells at each antibody concentration was subtracted from the total number bound to LK cells. Since the number of anti-L molecules bound to the LK cells from each animal is about the same it is unlikely that there are any antibodies present to antigens other than that associated with pump stimulation.

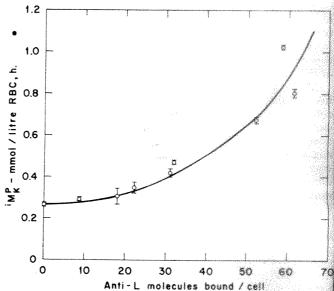


Fig. 2 The ouabain-sensitive K influx in LK cells  $(M_k^p)$  is plotted as a function of the number of anti-L molecules bound per cell; the data are from Fig. 1.

In Fig. 1 the number of antibody molecules bound to His cells and the number of corrected anti-L molecules bound to Lis cells is plotted as a function of the amount of labelled antibod to which the cells were exposed. The pump rate determined of the same LK cells is also plotted as a function of the amount of antibody. The number of molecules bound to HK cells is linear function of antibody concentration (as would be expected if this represents nonspecific binding), but the number of antimolecules bound to LK cells saturates at about 60 per cell which is close to the number (55) of pumps calculated from <sup>a</sup>H-ouabatic binding to the same cells<sup>a</sup>. As the number of anti-L inolecule bound approaches saturation, the stimulated pump rate als approaches saturation. It was not possible to determine the equilibrium constant for the antigen—antibody reaction since the eluted antibody contained large amounts of non-anti-L IgG.

In Fig. 2 the pump rate is plotted as a function of the number of antibody molecules bound. The stimulation of the pump rate is not a linear function of the number of antibody molecule bound; rather, the pump rate increases more rapidly at his antibody concentrations (high number of molecules bound than at low. This may reflect heterogeneity in the population of LK pumps so that the pumps with higher affinities for antibod are less stimulated than are those with lower affinities or it may indicate that more than one anti-L molecule per pump must be bound before the pump is stimulated. Since similar heterogeneity has been observed in the affinity of LK pumps for ouabain, the former possibility seems more likely.

Since the number of anti-L molecules bound to LK goat cel when the pump is maximally stimulated by anti-L (about 60) the same as the number of 3H-ouabain molecules bound by the same cells at 100% inhibition of the pump (about 55), it is concluded that anti-L combines with the pump (or with an antigen present in the same amount as the pump). Alteration of the kinetic characteristics of LK pumps by anti-L probably results from a conformational change induced in the pump by its combination with the antibody.

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#### Reaction of human smooth nuscle antibodies with human blood ymphocytes and lymphoid cell lines

ANTIBODIES to smooth muscle appear in serum from cases of ctive chronic hepatitis1,2 and transiently in various acute viral iseases3,4. The smooth muscle antibodies (SMA) are demontrated by indirect immunofluorescence (IFL) using cryostat ections of rat stomach and kidney and human thyroid5. The eaction of SMA-positive sera with lymphoid tissue has been ointed out6.7. Here we deal with the reaction of SMA in idirect IFL on human peripheral lymphocytes and especially with lymphoblastoid cell lines.

Lymphocytes were purified from defibrinated blood by gelatin edimentation. Phagocytic cells were removed with iron powder nd erythrocytes lysed by 0.83% NH<sub>4</sub>Cl solution. After washing a saline the lymphocytes were cultured with or without the ddition of phytohaemagglutinin (PHA) (Burroughs Wellcome), .5 μg per 106 cells ml-1, in RPMI-1640 medium enriched with 0% human AB serum. After 18 h culture, the medium conaining PHA was removed and new medium was added. Cell reparations were made at varying intervals as is shown in Table

After three washes in saline, the cells from each tube were suspended in 0.05 ml of 0.034 M sodium citrate, smeared, ried and fixed in dry acetone at -20° C. Preparations from our different donors were examined by IFL.

Cells from lymphoid cell lines were grown in suspension alture in RPMI-1640 medium supplemented with 15% foetal

Table 1 Immunofluorescence staining of surface microvilli on smears of cells with smooth muscle antibodies (SMA)

	Cells with short surface microvilli*	% Cells with long surface microvilli*
Moore (4 experiments)	27-64	3-20
MOLT-4 (3 experiments)	5-15	
HeLa	_	_
L cells	_	
Normal peripheral lymphocy	tes†	
before culturing	4	_
after 3 h in medium	4	
after 18 h in medium	7	_
after 42 h in medium	20	_
after 66 h in medium	8	_
after 3 h of PHA-stimular	tion 5‡ tion 43‡	
after 18 h of PHA-stimula	tion 43±	_
after 42 h of PHA-stimula	tion 53	1
after 66 h of PHA-stimula		4

Percentages are based on counting of 200 cells.

One representative experiment reported.

Most cells aggregated. Free-lying cells and cells in small aggregates counted.

calf serum. Two lines of Epstein-Barr virus (EBV) transformed lymphocytes, Robinson-7481 and Moore-7002, and one leukaemia cell line MOLT-4 (ref. 8), were tested. After three washes, cell smears were prepared in sodium citrate as described above. Cell preparations from nonlymphoid cell lines of human (HeLa cells) and mouse origin (L cells) were prepared in a similar manner. SMA-positive sera were allowed to react with the cell preparations using indirect IFL. The anti-human Igconjugate and the optic system used have been described earlier6.

Ten different SMA-positive sera were tested on lymphoblastoid cell lines and all gave the same type of reaction. Only the reactions of one representative serum 2888/73, will be reported here. This serum has been described earlier<sup>6</sup>. All SMA reactions of this serum were removed by absorption with an extract of smooth muscle from pig stomach5. Extracts for absorption were also made from lymphoblastoid cell lines, HeLa and L cells. One part of packed cells was mixed with two parts of phosphate-buffered saline and homogenised in an Omnimixer for 8 min. The supernatant obtained after centrifugation at 3,000 r.p.m. for 15 min was used as antigen for absorption. The

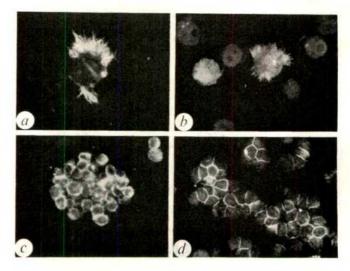


Fig. 1 a, Indirect immunofluorescence using SMA-positive serum on acetone fixed smear of a lymphoblastoid cell (Robinson-7481). Note the strongly fluorescent long microvilli. b, Indirect immunofluorescence using SMA-positive serum on acetone-fixed smear of lymphoblastoid cells (Moore-7002). Note one cell with cytoplasmic fluorescence and one cell with fluorescent microvilli and cytoplasmic fluorescence. c, Indirect immunofluorescence using SMA-positive serum on acetone fixed smears of peripheral blood lymphocytes cultured for 3 h with PHA. d, Indirect immunofluorescence using SMA-positive serum on acetone fixed smears of peripheral blood lymphocytes cultured for 3 h. (×295).

Table 2 Effect of absorption of SMA-positive serum 2888/73 with different organ or cell extracts

Absorption with extracts of:  Muscle layer of pig stomach:	Protein concentration (mg ml <sup>-1</sup> )	SM in rat stomach	Rat kidney	um titre agai Human thyroid membrane	nst: Mouse thymus	Smears of Moore cells	
undiluted diluted 1/4 Robinson cells Moore cells L cells HeLa cells Titre of unabsorbed serum	19.8 5.0 15.0 15.9 20.3 17.2	<10 <10 10 <10 160 160	<10 <10 10 <10 160 160	<10 <10 <10 <10 40 80 80	<10 <10 • <10 • <10 80 80 80	< 10 < 10 < 10 < 10 160 80 320	

antiserum 2888/73 was absorbed twice with equal volumes of the different cell extracts (Table 2).

A prerequisite for the demonstration of the microvilli of lymphoid cells was that the cells were suspended in 0.034 M sodium citrate before the preparations were made or that chelating agents were added.

SMA-positive serum showed by IFL a cytoplasmic or a membrane type reaction with lymphoblastoid cells. The most conspicuous finding was, however, the strongly fluorescent long 'hairy' microvilli extending from the surface of single cells (Fig. 1a and b), usually asymmetrically concentrated to one side of the cell. Most cells with long fluorescent microvilli showed very little cytoplasmic staining. The microvilli could reach the length of the diameter of the cell but they were usually shorter or around 1/3 of the diameter. The appearance of the surface microvilli seemed to vary somewhat with the cell cycle and with the growth conditions of the cells (Table 1). MOLT-4 cells had in comparison with cells belonging to the Moore or Robinson lines only short stub-like villi and fewer cells were positive (Table 1). Myeloma cells from an IgE producing line (obtained from Dr Kenneth Nilsson, Uppsala) showed no reaction with SMA.

Wet preparations of living lymphoblastoid cells were examined by phase contrast microscopy. Almost all cells had microvilli. Attempts to stain live suspended cells with SMA-positive serum by IFL were, however, negative.

Smeared and acetone fixed HeLa cells and L cells showed no microvilli stained with SMA-positive serum but a moderate or weak reaction of the peripheral part of the cytoplasm or of the membrane especially when the cells were in aggregates. This was considered to be a kind of contact phenomenon.

Absorption of serum 2888/73 with SM antigen from pig stomach removed all IFL staining of lymphoblastoid cells and also the SMA pattern on the tissue sections. The outcome of the absorption experiments is shown in Table 2. Extracts of Moore cells were almost as effective as extracts of the muscle layer of pig stomach. Extracts of HeLa cells and L cells hardly changed the titre of serum 2888.

When SMA-positive sera were set to react in IFL with smears of peripheral lymphocytes from normal blood donors a moderate reaction was observed with the cytoplasm or the membrane of 60-80% of the cells. About 5% of the cells had short villi (Table 1). When cells had been cultured for 3 h in a medium containing PHA, the cells in the aggregates formed were strongly positive (Fig. 1c). Cells cultured without PHA showed a weaker membrane-like staining (Fig. 1d) best visible when the cells were in close contact. At 18 h the IFL staining of the aggregated cells, cultured in the presence of PHA, was stronger than after 3 h. At 42 and 66 h many of the aggregates had broken up and many large and small cells with short microvilli could be seen (Table 1). Occasionally cells with long and asymmetric villi were observed resembling the lymphoblastoid cells described above. A similar increase in villi-containing cells has been observed in transmission EM after PHA stimulation11

'Hairy' cells have been observed earlier by phase contrast using wet preparations of cultured lymphoid cells, possibly pure B cell lines9. By scanning electron microscopy (SEM) lymphoid B

cells were shown to have long villi whereas T cells (MOLT-4 had small stub-like projections<sup>10</sup>. In the present investigation by IFL, the T cell line MOLT-4 and the probable B cell lines Moon and Robinson also showed differences in morphology, the lattel cells having more and longer villi staining with SMA-positive serum than had the MOLT-4 cells.

We have demonstrated that the SM antigen present in the peripheral lymphocytes increases considerably when the cellare stimulated by PHA. Surface microvilli appear stainable by SMA-positive serum. Such surface microvilli are present in high amounts in EBV-transformed lymphoblastoid cells. We sugges that the demonstrated SM antigen, probably consisting of contractile protein(s)12, in the microvilli are of biologica importance for the lymphoid cells, for example, in their mobility The cell lines Robinson-7481 and Moore-7002 were provide by Dr George E. Moore, Buffalo. This work was supported b the Swedish Cancer Society. K.L. received a grant from Ander Otto Swärds foundation.

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#### Replication of oncornavirus-like particle in human breast carcinoma cell line, MCF-7

THE search for RNA-containing human breast cancer virus has been helped by the discovery of several biochemical an biophysical characteristics of the oncornavirus group<sup>1</sup> Although these properties may not be unique to oncorn viruses<sup>5</sup>, they provide biological reagents for more discrimina ing tests<sup>6-8</sup> to establish species of origin, natural history ar virus taxonomy for candidate viruses exhibiting these pro-

Oncornavirus-like particles have been detected in human milk<sup>1-4</sup> and in breast carcinomas<sup>8,9</sup>. Evidence for oncornavirus infection in cultures of normal breast cells10, and breast carcinoma cells<sup>11</sup> has also been reported. To date, however, undefined cultural exigencies have precluded the general usefulness of established mammary carcinoma cell lines for growing particles to levels sufficient for characterisation as human breast cancer viruses. It should be noted that replication of the mouse mammary tumour virus is, in many cases, also severely restricted in monolayer cultures of mouse mammary tumour cells 12,13. This restriction can be largely prevented by cultivating mouse mammary tumour cells in conditions permissive for the expression of normal mammary cell differentiative functions<sup>12</sup>.

To obtain optimum culture conditions for the replication of putative human breast oncornavirus, MCF-7 human breast arcinoma cells were cultivated so as to maintain several activities of differentiated mammary epithelial cells. The presence of specific 17β oestradiol receptors and synthesis of & actalbumin (W. W. Rose and C. M. McG., unpublished) uggest the expression of differentiative features of mammary pithelium by MCF-7. MCF-7 is an established subtetraploid ell line originally derived from a pleural effusion of a female atient with malignant adenocarcinoma of the breast14. several criteria were used to establish the human nature of the cells<sup>14</sup>. These cells are free of mycoplasma<sup>14</sup>. Here we describe he isolation of an oncornavirus-like particle from MCF-7 cells and the results of immunofluorescence studies to characterise he particle serologically.

The density distribution of RNA-containing particles released rom MCF-7 cells during a 48 h 3H-uridine labelling period at 5°C is shown in Fig. 1a. The buoyant density of the major earticle population was determined to be 1.17 g ml<sup>-1</sup> ( $\pm 0.01$ ) in reformed sucrose gradients. A second, minor population, with density of 1.23-1.25 g ml<sup>-1</sup>, was also detected in some experiments. These particles, which represented 1-20% of the total a.p.m. in gradients, have yet to be fully characterised. When ells were labelled at 37° C instead of 25° C, however, only articles with densities of 1.23-1.25 g ml<sup>-1</sup> were recovered.

From the 1.16-1.18 g ml<sup>-1</sup> region of 15-65% sucrose gradients fifty different experiments  $5 \times 10^3 - 10 \times 10^3$  d.p.m. were ecovered. This corresponds to a yield of  $5 \times 10^3 - 10 \times 10^3$ p.m.  $734B/25 \times 10^6$  cells per 48 h in our labelling and purificaon conditions (Fig. 1a).

RNA was extracted from <sup>8</sup>H-uridine-labelled, isopycnicallyanded particles and was sized by gel electrophoresis. Four NA populations were identified in the 734B ( $\rho = 1.16-1.18$ ) article population (Fig. 1b). The major RNA species had a colecular weight of  $10 \times 10^6$ – $12 \times 10^6$ . The molecular weight of second heterogeneous population of RNA was  $\sim 3 \times 10^6$ - $\times$  10°. A third (high molecular weight) population ( $\sim 1 \times 10^6$ –  $2\times10^6$ ) coelectrophoresed with 28S (molecular weight  $1.75\times$ 106) ribosomal RNA from MCF-7 cells and probably reflected microsomal contamination in the gradient. The fourth population was heterogeneous low molecular weight RNA. Parallel sedimentation velocity analyses of 734B RNA also resolved four populations, 62-70S, 35-38S, 26-28S and 4-10S, which correspond well with molecular weight estimates of 734B RNA in sizing gels.

Particles isopycnically banded as above, were assayed for reverse transcriptase activity using endogenous RNA as template. The products of a 1 h 37° C reaction are shown in Fig. 2. The major RNA species consistently served as template for the polymerase reaction. Recovery of a discrete product (molecular weight  $3 \times 10^6 - 5 \times 10^6$ ) was variable but products

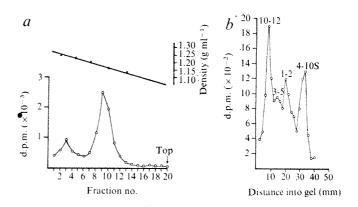


Fig. 1 a, Buoyant density of 734B particles in sucrose. Immediately before labelling  $25 \times 10^6$  MCF-7 cells with <sup>3</sup>H-uridine (15  $\mu$ Ci ml<sup>-1</sup>, New England Nuclear, specific activity = 20 Ci mol<sup>-1</sup>), growth medium (Eagle's MEM+10% calf serum (Flow Laboratories) + 10  $\mu$ g ml<sup>-1</sup> insulin) was replaced with MEM+5% heat inactivated (56°/30 min) calf serum+10  $\mu$ g ml<sup>-1</sup> insulin, and the incubation temperature lowered to 25°-29° C. After a 48 h label, particles were partially purified by a series of centrifugations in discontinuous and linear sucrose gradients<sup>13</sup> prepared in TNE buffer (0.01 M Tris-HCl, 0.15 M NaCl, 0.001 M EDTA, (pH 7.4)). Trichloroacetic acid precipitable <sup>3</sup>H-uridine d.p.m. in 0.2 ml fractions from linear 15-65% sucrose gradients after a  $14.1 \times 10^{6}g$  minimum centrifugation of particles to equilibrium is shown. Radioactive materials were counted using a Beckman model 3380 instrument calibrated to deliver absolute activity (d.p.m., which corresponds to 4-5×c.p.m. values). b, RNA extracted from 734B particles. Labelled particles released from  $75 \times 10^6$  cells were isopycnically banded as in Fig. 1. The 1.16-1.18 g ml<sup>-1</sup> region was pooled and pelleted in TNE buffer. RNA was extracted with sodium dodecyl sulphate (SDS) (0.75% final concentration) and phenol as described previously<sup>13</sup>. RNA was sized in 7.0 cm, 1.8% acrylamide/0.5% agarose composition gels<sup>25</sup> and mouse 28S (molecular weight 1.67×10°) cell RNA was used as an external marker. Each fraction represents 2 mm gel slices. Numbers over radioactive peaks represent apparent molecular weights in millions of daltons derived from the external marker.

Table 1 Reactivity of MCF-7 cells with anti-MuMTV and anti-MuLV sera in immunofluorescence tests

Market Control of the						
		I	Reactivity (% Cel	lls fluorescing)	1	
ntiserum	MCF-6	BALB/c cl-5	BALB/cfc3H	MCF-7w	MCF-7 cl-1	D-562
3AV*	0	0	40-75	5-7	0	0
3AV absorbed with MuLV†	0	0	4075	5-7	0	0
3AV absorbed with BALB/c cell1	0	ND	40-75	57	0	0
3AV absorbed with MuMTV§	0	ND	0.1 - 1.0	0	0	0
ormal rabbit serum	0	0	0	0	0	0
nti-MuLV	100	0	0	0	0	0

<sup>\*113</sup>AV was prepared in rabbits against MuMTV isopycnically banded from BALB/cfC3H milk. The serum was absorbed twice with BALB/c ilk, then absorbed overnight in female BALB/c mice before use. A 1:4 dilution of serum was used in each indirect immunofluorescence test<sup>24</sup> †MuLV grown in BALB/c spleen cells (MCF-6).

BALB/c cell is a lyophilised preparation of homogenised BALB/c mammary glands.

SMuMTV for absorption was isopycnically banded from BALB/cfC3H milk. Three absorptions were required to reduce reactivity of the rum from BALB/cfC3H cells to 0.1-1.0%

Anti-MuLV was obtained from Flow Laboratories and contained both anti-gs-1 and anti-gs-3 reactivity20. The serum was used at a 1:100

Cells used: MCF-6, a leukaemic BALB/c spleen cell line<sup>19</sup>; BALB/c, a cloned derivative of a BALB/c mammary tumour cell line<sup>27</sup>; BALB/C3H, Primary cultures of BALB/cfC3H mammary tumour cells<sup>13</sup>; MCF-7w, uncloned cultures of MCF-7 cells<sup>14</sup>; MCF-7 cl-1, a cloned subline MCF-7; D-562, a human cell line derived from a pleural effusion of a patient with a throat adenocarcinoma18.

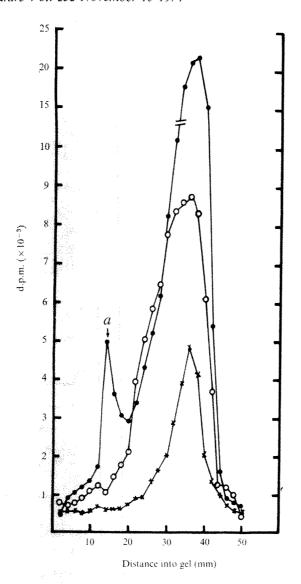


Fig. 2 Endogenous reverse transcriptase activity of 734B particles. Unlabelled particles released from 100 × 106 cells were isopycnically banded as for Fig. 1. The 1.16–1.18 g ml<sup>-1</sup> region was pooled and pelleted through a cushion of 15% sucrose in TNM buffer, pH 7.4 (Tris-HCl [0.01 M], NaCl [0.15 M], MgCl<sub>2</sub> [0.003 M]). Reverse transcriptase assays were performed<sup>1</sup>. The incubation mixture consisted of 734B protein (130 μg), NP-40 (0.2%), DTT (0.033%), KCl (0.04 M), MgCl<sub>2</sub> (0.006 M), dATP, dGTP, dCTP (0.8 μmol ml<sup>-1</sup> for each) and <sup>3</sup>H-TTP (100 μCi, specific activity = 40 Ci mmol<sup>-1</sup>). Reactions at 37° C were terminated after 1 h by addition of NaCl (to 0.4 M), SDS (to 1%) and phenol (to 50%). Phenol extracted products were sized in 1.8% acrylamide/0.5% agarose gels<sup>25</sup> and the molecular weight of products was determined from the position of an external 1.67 × 106 molecular weight marker (ribosomal 28S RNA). The major product (molecular weight 10 × 106–12 × 106) corresponds to the radioactivity in the peak under a. • Products from a 1 h standard reaction<sup>1</sup>; ○, products from a 1 h reaction incubated with pancreatic RNase (10 μg ml<sup>-1</sup>) for 15 min at 37° C before addition of nucleoside triphosphates; ×, products from a 1 h reaction in which deoxyguanosine triphosphate had been omitted from the reaction mixture. 734B protein (43 μg) was included in all three reactions.

ranging in size from  $4 \times 10^6$  to small (4S) fragments were routinely recovered in a single heterogeneous peak which, when integrated, accounted for the vast majority of  $^3$ H-TPP c.p.m. after 1 h enzyme reaction.

Preincubation of the NP-40-disrupted 734B with pancreatic ribonuclease (10  $\mu$ g ml<sup>-1</sup> at 37°C) virtually eliminated the <sup>3</sup>H-TTP counts in the  $10\times10^6$ – $12\times10^6$  molecular weight region of sizing gels (Fig. 2) indicating that the high molecular weight RNA served either as template or primer in the reaction. The

lower molecular weight products were reduced by approximately 65%. Omission of deoxyguanosine triphosphate from the reaction mix also eliminated the  $^{8}$ H-TTP c.p.m. from the  $10 \times 10^{6}$ - $12 \times 10^{6}$  molecular weight region of the ge (Fig. 2), and reduced the smaller molecular weight produce by approximately 85%. This indicates synthesis of a propenter opportunity of high molecular weight RNA-DNA rather than terminal addition of  $^{8}$ H-TTP to RNA by a terminal transferase.

Analysis of the reaction products in CsSO<sub>4</sub> gradients ind cated that 15–30% of the total product after 1 h incubatio consisted of RNA-DNA hybrids with a density range (1.68–1.55 g ml<sup>-1</sup> (Fig. 3). The remainder consisted of DN (density ~1.45, Fig. 3). That the RNA-DNA product was hydrogen bonded heteroduplex rather than a covalently linke polynucleotide was shown by denaturing the product if formamide<sup>17</sup> before analysis in CsSO<sub>4</sub> gradients (Fig. 3). Suc treatment resulted in removal of the <sup>3</sup>H-TTP counts from the RNA and hybrid regions of the gradient and recovery of the counts in the DNA region of the gradient (Fig. 3).

An antiserum against murine mammary tumour viru (MuMTV) (prepared against isopycnically banded virus from BALB/cfC3H mouse milk) reacted in indirect immunofluores cence tests with 5-7% of MCF-7 cells (Table 1). Granula fluorescence was primarily localised in the cytoplasm of staine cells. A cloned derivative of the human cell line which ha exhibited no evidence of 734B particle release (MCF-7 CIwith no reverse transcriptase activity in a 1.16-1.18 g ml gradient band) showed no reactivity with the anti-MuMT serum (Table 1). Similarly, another human epithelial cell lin (D-562), also derived from a pleural effusion<sup>18</sup>, and not asso ciated with particle synthesis, showed no reactivity with the anti-MuMTV serum (Table 1). The antiserum did not reac with BALB/c mouse cells infected with murine leukaemi viruses (MuLV) nor with MuMTV-free BALB/c mouse man mary epithelial cells27 (Table 1).

Absorption of this antiserum with either MuMTV-fre BALB/c mouse milk, MuLV grown in BALB/c cells or extract of BALB/c mammary glands had no effect on its reactivity with either MuMTV positive mouse cells in primary culture (BALB/cfC3H) nor with human MCF-7 cells (Table 1). It contrast, absorption with MuMTV isopycnically banded from

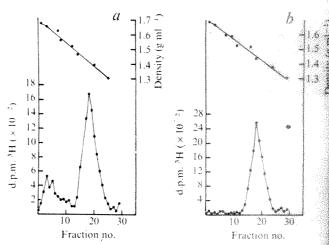


Fig. 3 Density of reverse transcriptase products in CsSO<sub>4</sub> gradients. Ethanol precipitated, SDS-phenol extracted product of a 1 h reverse transcriptase reaction (compare legend for Fig. 2) was further purified by the CTAB method<sup>28</sup>. The product reconstituted in  $0.5 \times SSC$  buffer and 50% formamide<sup>15</sup> (final concentrations), was divided into two equal aliquots. One half was heated at  $80^{\circ}$  C for 10 min, conditions adequate to melt RNA-DNA hybrid polymers<sup>17</sup>. Both heated and unheated products were dialysed against 0.001 M Tris buffer pH 7.0, mixed with CsSO<sub>4</sub> (p=1.51) and centrifuged 60 h at 45 K in a 50 Trotor at 25° C. a, Unheated product; b, product heated at  $80^{\circ}$  C

mouse milk resulted in a marked reduction of its reactivity with both BALB/cfC3H mouse cells and with MCF-7 cells (Table 1).

An antiserum against MuLV protein, reactive against both inter- and intraspecies MuLV group determinants 20, which in immunofluorescence tests was reactive to a dilution of 1:2,000 for MuLV positive mouse cells, was unreactive with MCF-7 cells even at a dilution of 1:100 (Table 1).

Thus, 734B particles, with a buoyant density of 1.16-1.18 g ml-1, a high molecular weight RNA and a bona fide reverse transcriptase enzyme seem to be antigenically unrelated to MuLV. This is consistent with results of biological tests in which concentrates of 734B particles and disrupted MCF-7 cells failed to induce leukaemia (or any other overt disease) in BALB/c mice. Similarly, 734B particles did not activate defective sarcoma virus in S+L- NIH Swiss 3T3 (ref. 22) cells.

The interpretation of the antigenic relationship between 734B and MuMTV is less clear. Studies of Black and his colleagues23 have suggested immunological cross reactivity between MuMTV and an agent in human breast carcinoma cells. Likewise, Axel et al.8 have suggested homology between MuMTV RNA and RNA from oncornavirus-like particles in human breast tumours. In our own studies, reactivity of 734B-synthesising MCF-7 cells with an anti-MuMTV serum (Table 1) coupled with the fact that the reactivity for MCF-7 cells could be absorbed from anti-MuMTV sera with purified 734B (C. M. McG., P. M. G., H. D. S., T. G., and M. A. R., unpublished), suggests an antigenic relatedness between MuMTV and 734B. Preliminary results of radioimmunoassays and protein sizing studies, however, suggest significant structural differences between MuMTV and 734B. Studies currently in progress in our laboratory to identify the protein(s) of 734B reacting with anti-MuMTV sera and to evaluate the homology between the RNAs from the two agents are aimed at the ducidation of their true relationship.

Definitive morphological characterisation of 734B requires nore particles than are synthesised in existing cultural condiions. We are now attempting to stimulate 734B particle production to reproducibly quantifiable levels by altering those conditions.

We are also actively engaged in experiments to establish species of origin for 734B as well as the natural history of 734B expression in normal breast and breast carcinoma cells using he DNA product of the reverse transcriptase reaction as a probe'6,21 for 734B information. This data is of obvious undamental importance for an understanding of the relationhip of 734B to human breast cancer.

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#### Unique increase of serum proteins and action of antitumour polysaccharides

WE have reported that lentinan and several other polysaccharides inhibited the growth of sarcoma-180 transplanted subcutaneously in mice1-4, and that their antitumour activity is a host-mediated reaction with participation of the thymus or thymus-dependent cells (T cells)5-8. While it has recently been reported that lentinan is a T cell oriented adjuvant9 and a helper T cell stimulant10-11, it is not clear how lentinan affects the host at the first stage before the induction of many immunological changes. It is possible that the antitumour activity of lentinan is dependent on the presence of certain substances in the host which interact with these polysaccharides.

We have found that three kinds of protein components, which are different from properdin, increase markedly in mouse serum soon after lentinan administration. It should also be noted that there is a close relationship between the increase of protein components and the antitumour activity of polysaccharides.

ICR female mice (body weight 20-25 g) were given a daily intraperitoneal injection of 4 mg per kg lentinan (the optimal dose for tumour regression) for 5 d. Serum was obtained conventionally from blood on days 1, 4, 7, 10, 14, and 21 after the final injection of lentinan. A 5 µl aliquot of serum obtained on each of these days was applied to polyacrylamide gel electrophoresis on gradient gel PAA 4/30 (Pharmacia Fine Chemicals, Uppsala) at 125 V for 15 h using 5 mM Tris-glycine buffer (pH 8.6). The gel was stained with 1% solution of Amidoblack 10B, followed by destaining in 7% solution of acetic acid.

The results of electrophoresis show that mouse serum proteins were divided into 21 fractions (Fig. 1). It is apparent that three protein components (LA, LB, and LC) markedly increase in

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Table 1 Unique increase of serum protein, LA, LB, and LC, after the administration of antitumour polysaccharides

					management of the state of the		
Polysaccharides	Dose	In	Increase of serum proteins				Tumour
Lentinan Pachymaran CM-pachymaran† Zymosan Control (saline) Pachyman	$\begin{array}{ccc} (\text{mg kg}^{-1} \times 5) & \text{LA} \\ & 4 & +++ \\ 10 & ++ \\ 75 & ++ \\ 20 & ++ \\ 0.1  (\text{ml}) & \end{array}$	Date of peak*  4  7  7  7	LB +++ +++ +++	Date of peak 4 7 7 7	LC +++ +++ +++	Date of peak 7 10 7	inhibition ratio (%) 98.0 95.3 98.4 91.5
Laminarin	$\begin{array}{cccc} 10 & + \\ 10 & - \end{array}$	1	++	1		law.	- 7.0
Starch Dextrant	10 —		manus.	Mindry Mindry	MANNA.	and and a second	- 7.0 Not effective
Cellulose	10 —		-	delenan	**************************************	dellerede	-21.2
			******	*******	-Attracts	gingspan (h)	10.1

The mice were ICR female mice.

Date of peak was counted after the last injection of polysaccharides.

Carboxymethylpachymaran.

Molecular weight 200,000-275,000.

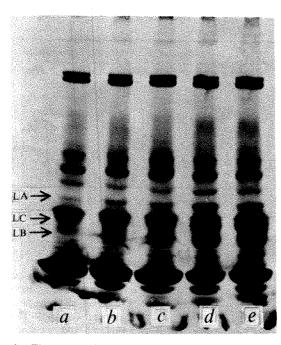


Fig. 1 The acrylamide gel pattern of serum samples from lentinan-treated mice. a, Control (fourth day after saline injection); b, first day; c, third day; d, fourth day; e, seventh day after the last injection of lentinan.

the serum of mice administered lentinan. Their increase peaked on days 4 to 7 after the final injection of lentinan, then gradually decreased, and the contents of LA, LB, and LC on day 21 were not different from those in control mice. Quantitative measurement of serum proteins with a densitometer showed that serum proteins LA, LB and LC on the fourth day after the last lentinan injection were 2.8, 2.4, and 2.1 times as much as those in the control serum. The ratio of LA, LB, and LC content to the whole serum contents was 6.2%, 10.3%, and 11.3%, respectively. Although the complete assignment of serum components fractionated by this method is not yet established. we consider that LA is  $\beta$  globin and LB and LC are  $\alpha$  globins.

Further, we studied the correlation between the increase of serum protein components and the antitumour activity of polysaccharides. The alteration in the content of serum proteins LA, LB and LC in mice administered polysaccharides with or without the activity was compared, the method being identical to that used with lentinan. These results are summarised in Table 1. There was an increase of serum protein LA, LB, and LC in mice administered antitumour polysaccharides, such as lentinan or pachymaran, even though the duration of peak varied. On the other hand, changes of serum protein LA, LB, and LC in mice given polysaccharides without antitumour

activity was not significantly different from those in control mice, except for pachyman.

Pillemer et al., reported that properdin, a B globulin, which was adsorbed from serum by incubation with zymosan, increased in titre 200-300% above the normal level 2-14 d after the intravenous injection of zymosan12-14. Serum protein LA, LB, and LC, however, were not adsorbed with zymosan or lentinan by Pillemer's method. In addition, there is much more LA, LB, and LC than properdin in serum. These findings seem to indicate that none of proteins LA, LB, and LC is properdin.

That the antitumour polysaccharides have an anticomplementary activity in vitro15, and that they affect a-helix content of serum albumin<sup>16</sup>, indicates that in an early stage, serum factors play an important role in tumour regression, as well as in cell-mediated immune responses. We consider that there may be a close relationship between the drastic increase of the proteins (LA, LB, and LC) and a potent stimulation of T helper cells<sup>11</sup>. Although the relation between these serum proteins (LA, LB, and LC) and the host defence mechanism against tumours remains obscure, the dramatic increase of these proteins by antitumour polysaccharides may indicate a new approach to the study of antitumour immunity.

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#### Liposomes as immunological adjuvants

ADJUVANTS are widely used to increase antibody formation in experimental animals, for example, Freund's incomplete adjuvant, a water-in-oil emulsion containing the antigen, and Freund's complete adjuvant, which is the same but with killed tubercle bacilli. These adjuvants cannot be used in man because the mineral oil base is not degraded and persists at the injection site. Particularly with the complete adjuvant, unacceptable granulomas can be formed. There is real need for a safe and effective adjuvant for use in human immunisation programmes.

Such an adjuvant could reduce the amounts of antigens,

which is administered to humans, can be conveniently labelled with radioactive iodine and is readily incorporated into liposomes; the formation of antibodies can be measured easily by passive haemagglutination. Representative observations in mice immunised with free and liposome-entrapped DT by the intravenous and subcutaneous routes are presented in Table 1. DT administered intravenously in negatively-charged liposomes elicits the formation of much higher concentrations of antibodies than are elicited by free antigen. Subcutaneous (footpad) inoculation of DT in negatively-charged liposomes elicits significantly higher primary and secondary antibody responses than inoculations of the same doses of free antigen. In contrast, antigen entrapped in positively-charged liposomes elicits less antibody than the same dose of free antigen.

Intramuscular injection of DT in negatively-charged liposomes again elicits significantly higher antibody levels than free DT (Table 1). In these and other experiments, dicetylphosphate-containing liposomes as adjuvants were superior to those containing phosphatidic acid. When immune mice were inoculated intravenously with free DT they developed severe

Table 1 Serum antibody responses of mice to diphtheria toxoid administered free or in liposomes of different compositions

Experimental group	No. of mice	Mode of administration	Route of adminstration	Primary Ab resopnse	Secondary Ab response	Probability
a	15	Free	Intravenous	1.8	-	a v b, P < 0.01
b	15	-Liposomes (PA)	Intravenous	10.0	w/materials	
c	6	Free	Subcutaneous	2.5	11.6	c v d, P < 0.01
d	6	-Liposomes (PA)	Subcutaneous	6.7	13.3	d v e, P < 0.01
e	6	+ Liposomes	Subcutaneous	0	11.3	c v e, P < 0.10
f	6	Free	Intramuscular	1.7	7.5	f v g, P < 0.10
g	6	-Liposomes (PA)	Intramuscular	3.7	11.0	g v h, P < 0.05
ĥ	6	-Liposomes (DP)	Intramuscular	6.6	12.0	f v h, P < 0.01

Mice were of the TO strain, except in groups c-e, which were of the CBA strain. Positive (+) liposomes (0.5 mg lipid) were composed of egg lecithin, cholesterol and stearylamine in molar ratios 7:2:1; in negative (-) liposomes phosphatidic acid (PA) or dicetylphosphate (DP) replaced stearylamine. Primary serum antibody responses were measured after 13 or 14 d, booster injections with 20 µg antigen in the same form were given and secondary responses were measured after a further 10 d (groups c-e) or 14 d (groups f-h). Antibody responses were measured by indirect haemagglutination (ref. 4) and expressed as the log<sub>2</sub> IH titre.

for example, diphtheria and tetanus toxoids, required for immunisation, with corresponding economies especially relevant to the developing countries. Live virus vaccines have hazards such as the potential initiation of persistent infections or malignancy, and vaccines containing killed viruses or viral antigens often require adjuvants to elicit the formation of protective antibodies. Another possible danger of the use of adjuvants is that in a minority of recipients they may induce allergic reactions to the vaccines which they contain, especially of antigens are administered twice to boost immunity, or if the recipients are already sensitised to an antigen at the time of its administration. It would be an advantage if the mode of presentation of antigens in the adjuvant were such as to avoid allergic reactions. If adjuvants consist of immunogenic materials such as glycolipids or proteins, even in trace amounts, they night themselves induce allergic or even autoallergic reactions through sharing of antigens with those of the recipient or some other mechanism.

We report here the use of liposomes, concentric spheres consisting of phospholipid bilayers separated by aqueous compartments, as immunological adjuvants. Protein and other antigens can be entrapped in liposomes, which have been proposed as carriers of therapeutic agents<sup>1,2</sup>, and we have ound that under certain conditions the administration of entigens so entrapped elicits the formation of more antibodies han are elicited by the same doses of free antigen. Liposomes an be made of non-immunogenic, biodegradable materials and their composition and properties such as electric charge an be varied at will. If animals which already have antibodies re challenged with antigens entrapped in liposomes they are nuch less likely to develop antibody-mediated allergic reactions han when challenged with the same doses of free antigens3.

Diphtheria toxoid (DT) was studied because it is an antigen

serum sickness reactions, and the majority died. In contrast, the same amount of antigen inoculated subcutaneously or intravenously did not elicit Arthus or serum sickness reactions<sup>3</sup>. The investigations which we have carried out show that liposomes are potentially useful as adjuvants and there is reason to believe that further modifications of the system, such as the incorporation of Bordetella pertussis, Mycobacterium tuberculosis or microbiological or chemical products known to have adjuvant activity, will further increase their usefulness in practice.

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#### Differences in the effects of adult thymectomy on T-cell mediated responses in vitro

EVIDENCE exists indicating that functionally different subsets of T lymphocytes are activated during the in vitro allograft response. Thus, the T cells responding primarily by proliferation in the mixed lymphocyte culture (MLC) may be distinct from the T cells which differentiate to killer cells and display specific target cell lysis  $(CML)^{1-3}$ . The biological properties of the T-cell subsets involved are incompletely known. Adult thymectomy (ATX) in mice and rats alters certain T lymphocyte mediated functions. Spleen T cells forming rosettes with sheep erythrocytes disappear as soon as 5 d after ATX <sup>4.5</sup>. Lymphocyte with high amounts of surface  $\theta$  antigen are depleted from the spleen within a few weeks after the operation (Ref. 6 and C. Fournier and J. F. B., unpublished).

These early changes in the T-cell functions following ATX have been taken to indicate the existence of a newly formed, shortlived T lymphocyte population in the spleen regulated by the intact thymus either through a continuous cell traffic from the thymus or through the action of a thymic hormone? We have investigated whether the proliferating and lytic functions in the MLC-CML may be distinguished by selective depletion of a thymus-dependent (T) cell population by ATX, and also tested the effect of ATX on the *in vitro* responses of mouse spleen cells to the T mitogens, phytohaemagglutinin (PHA) and concanavalin A (con A). The results indicate that the killer cell precursors are more dependent on intact thymus function than the precursors of proliferating cells.

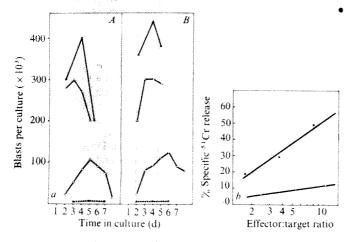


Fig. 1 a, Responses of CBA/H spleen lymphocytes to DBA/2 mitomycin-blocked spleen cells (○), PHA (△) and con A (■) 56 d after adult thymectomy. -•-•, background responses of non-stimulated CBA/H cells. A, lymphocytes of ATX mice; B, lymphocytes of STX controls. b, Lysis of P-815 mastocytoma cells by CBA/H + DBA/2m-primed cells on the day 7 of culture in the MLC. ○, MLC responder cells from ATX mice; ●, responder cells from STX controls (10,000 target cells, exposure time 6 h).

CBA/H-T6T6 or CBA/H mice were thymectomised by suction at 5 and 6 weeks of age. Age- and sex-matched controls were either sham-thymectomised (STX) or left untreated. Three separate experiments, each including 30-80 ATX mice were performed. Three to ninety days after the thymectomy the spleens were removed, spleen cells of three to five mice in each group were pooled and the lymphocyte-enriched population of the spleen cells (yielding approximately 85% of lymphocytes) was processed as described<sup>8</sup>. The spleen lymphocytes were then passed through nylon-wool columns9 to remove most of the non-T cells. After the procedure, 8-15% of the cells were found to carry surface immunoglobulin detectable by immunofluorescence with FITC-conjugated sheep anti-mouse Ig; 1.5 imes106 spleen lymphocytes from each group were cultured with optimal concentrations of PHA-M (Difco Pharmaceuticals, Detroit, final dilution 1.150) or con A (final dilution 10 µg<sup>-1</sup> ml) in 2ml of Eagle's minimal essential medium (Orion Pharmaceuticals, Helsinki), substituted with 5% of heat-inactivated foetal calf serum from selected batches (Flow Laboratories, Irvine, Scotland) in 11.5  $\times$  100 mm round bottom test tubes (Münnerstadt Glasswahrenfabrik, Münnerstadt) in a humidified atmosphere of 5% CO<sub>2</sub> in air<sup>8</sup>. Alternatively, the spleen lymphocytes were stimulated by 3.0 imes 106 mitomycin-C blocked DBA/2

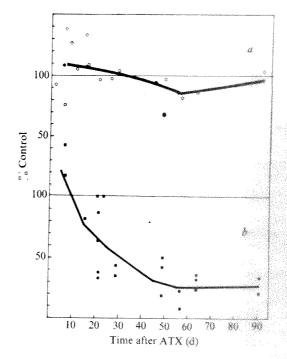


Fig. 2 Responses of CBA spleen lymphocytes to allogeneic lymphocytes (DBA/2m) in MLC (a) and lysis of P-815 (DBA/2) mastocytoma cells by MLC-primed cells (b), 3 - 90 d after adult thymectomy. All responses given as % responses of shamoperated control spleen cells tested on the same day. For detail see text.

cells (referred to in the text as DBA/2m) in conditions where only T cells are responsive<sup>10,11</sup>. On day 7 or 8 of culture, the MLC-primed cells were tested for the ability to lyse relevant <sup>51</sup>Cr-labelled P-815 mastocytoma target cells. In the cell-mediated lysis (CML) test<sup>8</sup> standardised conditions and 6 h exposure times were employed. Specific <sup>51</sup>Cr release was calculated according to Brunner<sup>12</sup>, the background release being in all experiments less than 15% of maximal release.

Examples of typical mitogen, MLC and CML responses by lymphocytes from thymectomised and sham-thymectomised mice in a single test (56 d after the thymectomy) are given in Fig. 1a and b. Fifty six days after ATX, the mitogen and MLC responses were close to sham-operated controls, whereas the CML response was clearly depressed. As responses of spleen lymphocytes obtained from non-treated control mice did not significantly deviate from sham-operated controls, the nontreated controls have not been included in the results. Figure 2 summarises the pooled data of the three separate experiments As regards the mitogen and MLC responses, only maximal responses, regardless whether they take place on the same day or not, have been considered. The responses of lymphocytes obtained from thymectomised mice are expressed as percentage responses of lymphocytes obtained from sham-operated controls. Spleen lymphocytes from ATX mice responded less efficiently to PHA from week 3 onwards after the thymectomy, The responses remained depressed for approximately 8 week but then returned back to control level. A similar depression taking place in approximately equal time period after ATX, was observed in the responses of spleen lymphocytes to con A. The depression, however, was not as remarkable as the depression of the response to PHA. A slight depression in the response of spleen lymphocytes to allogeneic cells was observed 7-9 week after adult thymectomy. This was in marked contrast with the effect of ATX on the CML response by MLC-primed cells The ability of in vitro primed cells to lyse relevant allogeness target cells was possibly first increased (up to 2 weeks ATX) but markedly depressed from the week 3 onwards, and remained depressed to approximately 30% of the control level throughout the observation period of 90 d.

The depression of T-cell responsiveness to phytohaemagglutinin, con A and in the MLC and CML reactions may be explained by a decrease in the relative amount of T cells in the spleen, especially since the percentage of spleen  $\theta$ -positive cells decreases after ATX (refs 4 and 6 and C. Fournier and J. F. B., unpublished). The proportional decrease in the number of T cells, however, cannot explain our results since first, the responder cells have been passed through nylon columns before being used, which is known to remove macrophages and most non-T cells9 and, second, there is an obvious dissociation between the various T-cell functions quantitated. The enrichment of T cells afforded by nylon-wool filtration could explain the relaitively low depression of the proliferative response in the MLC found in this study as compared to earlier reports 13,14 (which in addition concerned two-way MLC).

Our results, as regards the temporary depression of T-cell mitogen responses following ATX, confirm the findings by Folch and Waksman on the effect of ATX on T-cell mitogen responses in the rat15. Our findings show a dissociation in the effects of ATX on the MLC and CML responses. The CML response seems to be more dependent on an intact thymus function than the MLC response. Howe has shown that anti-0 serum plus complement inhibited CML responses more readily than MLC responses, suggesting that the killer cell precursors had a larger amount of  $\theta$  antigen on their surface than the MLC proliferating cell precursors 16. Since ATX selectively depletes those spleen cells which express a high amount of  $\theta$  antigen on their surface (C. Fournier and J. F. B., unpublished), it may be that these cells are the precursors of CML effector cells.

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#### Ganglioside catabolism in hexosaminidase A-deficient adults

TAY-SACHS disease is an inborn error of glycosphingolipid metabolism characterised by the accumulation of N-acetylgalactosaminyl - (N-acetylneuraminyl) - galactosylglucosylcera mide (G<sub>M2</sub>) and N-acetylgalactosaminylgalactosylglucosylceramide (GA2) in various tissues of affected individuals1. A defect in the removal of N-acetylgalactosamine from G<sub>M2</sub>, catalysed by a hexosaminidase, has been demonstrated as the underlying deficiency in all forms of G<sub>M2</sub>-gangliosidosis<sup>2,3</sup>. In Tay-Sachs disease this defect has been associated with the absence of one (the A form) of the two major isozymes of β-N-acetylhexosaminidase (Hex A and Hex B)4. Hex A can be differentiated from Hex B in serum, leukocytes, cultured skin and amniotic fluid cells, and urine by using various physical characteristics of these enzymes<sup>5</sup>. The smaller proportion of A relative to B is the basis of screening for heterozygous carriers of Tay-Sachs disease<sup>6</sup>.

A deficiency of Hex A has been demonstrated in the healthy members of a family where other members have had Tay-Sachs disease7.8 and this is difficult to reconcile with the currently accepted interpretation of the absence of Hex A in Tay-Sachs disease. It has been suggested that this relationship can be clarified by studying the hydrolysis of labelled  $G_{\text{M2}}$  and  $G_{\text{A2}}$ in appropriate tissues from these clinically normal A-deficient adults. We report here the catalytic properties of leukocyte hexosaminidases from such individuals when the natural substrates,  $G_{M2}$  and  $G_{A2}$ , are used to measure activity. These levels are compared with those present in normal A-containing adults and Tay-Sachs heterozygotes and homozygotes.

Leukocytes were prepared from 20 ml of blood as follows9. Red cells were allowed to settle from whole blood at room temperature in a heparin-dextran solution. Plasma containing leukocytes and platelets was removed and centrifuged at 1,000g for 10 min. The pellet, containing white cells, was removed and subjected to osmotic shock to remove residual red cells and then recentrifuged. The resulting pellet was washed twice with physiological saline. The final white cell pellet was homogenised in 2.5 ml of 0.25 M sucrose in a tight-fitting all glass homogeniser and the homogenate was centrifuged in the cold at 1,500g. The supernatant was removed and used as the source of enzyme. Incubations were carried out with the substrate 4-methylumbelliferyl-β-N-acetylglucosamine7 or the specifically labelled natural substrates G<sub>M2</sub> and  $G_{A2}^{3}$  with the modifications noted in Table 1.

Leukocyte preparations from normal controls, Tay-Sachs heterozygotes and A-deficient adults all possessed enzymes able to degrade the artificial and natural substrates. Leukocytes from the Tay-Sachs homozygote possessed no catalytic activity toward  $G_{M2}$  as substrate but could degrade  $G_{A2}$ . Although the specific activity of G<sub>M2</sub> hydrolysis in the Tay-Sachs heterozygotes was lower than the A-deficient adults, and probably indicates some differences in the enzymes' active site, this difference in specific activity by itself may not be significant. There were no differences in the specific activities of the groups when artificial substrate was used to monitor total hexosaminidase activity. As previously shown, using the artificial substrate7 the A-deficient adults and Tay-Sachs homozygotes were phenotypically the same in their deficiency of the A form. All samples studied had catalytic activity toward GA2 with overlap in the specific activities between groups. By consideration of the ratio of G<sub>M2</sub> activity to G<sub>A2</sub> activity, it was possible to classify the heterozygotes as having a ratio of about one half of the normal controls. This type of classification is valid as no deficiency in GA2 hydrolysis is reported in Tay-Sachs homozygotes. When the same ratio was calculated for the A-deficient adults, similarities to the Tay-Sachs heterozygotes rather than to normal controls were obtained. It is believed, however, that these two heterozygous states may be fundamentally different as the lower G<sub>M2</sub>/G<sub>A2</sub> ratio in classic Tay-Sachs carriers seems to be due to a decrease in  $G_{M2}$  hydrolysing

Table 1 Artificial and natural substrate hydrolysis in leukocytes from normal and various A-deficient persons

Source	4-MU-N-acetylglucosaminic	Hexosaminidase activities  4-MU-N-acetylglucosaminide* G <sub>M2</sub> † (μmol mg <sup>-1</sup> protein <sup>-1</sup> ) (pmol mg <sup>-1</sup> protein <sup>-1</sup> )		
Normals 1	4.3	200		(pmol nmol -1)
2	4.6	230	3.8	53
3			6.4	36
Tay-Sachs homozygote	5.2	310	8.9	35
Tay-Sachs heterozygotes	4.8	0	10.7 •	Spelander
1	2.4	90	5.2	17
2	4.4	120	6.3	19 18
3	5.1	150	8.2	18
A-deficient adults	<b></b>	150	0.2	10
1	4.0	180	9.8	18
2	4.8	190	16.7	1.1
3	4.8	220	15.2	14
Parents of A-deficient adults	7.0	220	1.2.4	2.4
Mother	3.2	170	11.3	1.6
Father	4.6	280	9.7	29

<sup>\*</sup>See ref. 7.

activity, while in the A-deficient adults there is a combination of lower G<sub>M2</sub> and higher G<sub>A2</sub> hydrolysing activity. It was of interest to study the parents of the A-deficient adults who appear as Tay-Sachs heterozygotes by conventional criteria, to determine whether they might be classified by the evaluation of  $G_{\text{M2}}$  to  $G_{\text{A2}}$  activity. The mother with a ratio of 15 seems to be a true Tay-Sachs heterozygote; the father possesses an intermediate ratio, 29, and seems to be the carrier of a new mutation which was previously postulated by pedigree analysis.

It has been generally assumed that only Hex A is involved in  $G_{M2}$  degradation<sup>10</sup>. Significant amounts of  $G_{M2}$ -hydrolytic activity, however, have been demonstrated in homogeneous preparations of either Hex A or B from human placenta11. In these preparations, the kinetic properties of both hexosaminidases with this substrate were essentially identical. Although it is difficult to extrapolate from these data to the situation in vivo, the possibility that Hex B normally participates in G<sub>M2</sub> catabolism must be considered. If this is indeed the situation, then the loss of the A form and existence of individuals with no A should be expected. One of the correlates of this interpretation is the necessity of an abnormality in Tay-Sachs patients of at least the part of the hexosaminidase B component involved in G<sub>M2</sub> degradation. The data presented here are consistent with both the genetic model previously postulated7 and with the model for hexosaminidase relationship already proposed11,12 where A is a conformer of B or represents an independent modification of the B gene product.

These findings are also consistent with the common and unique subunit theory for the relation of Hex A and B13 but are not readily reconcilable to the specifier subunit theory13.

Finally, as long as enzymatic activity with artificial substrates alone is used to classify carriers and patients with Tay-Sachs disease<sup>5</sup>, it will be impossible to distinguish both the A-deficient adults from those with Tay-Sachs disease and their parents from typical Tay-Sachs carriers. The frequency of this subpopulation among Tay-Sachs carriers is not known and based on current methods of prenatal diagnosis14, the A-deficient adults would today be classified as Tay-Sachs patients.

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#### Specific binding of growth hormone to thymocytes

As well as its general trophic effect on lymphoid tissue growth hormone (STH) specifically increases the activity of thymocytes in a graft versus host reaction and their helpe activity3. In vitro studies showed that STH alters the mitoti activity, RNA and protein metabolism of thymocytes\* At least for the effects obtained in vitro, it is assumed the STH binds to the thymocytes and thereby changes their physiological activity. The nature of this binding has no been studied. To learn about the characteristics of the surmised interaction, bovine STH (Nutritional Biochemical Cleveland, Ohio) and bovine prolactin (National Institute of Health, lot NIH B4) were labelled with 128 I and the binding to lymphoid cells was studied.

The labelling mixture contained 3 mg hormone, 200 µ lactoperoxidase (Sigma Lot No. 112c-0320), 100 μg glucose oxidase (Worthington, GOP 2 LA, 105 IU mg<sup>-1</sup>), 5 m glucose, 5×10<sup>-7</sup> M KI and 5 mCi carrier-free <sup>128</sup>I (Eidgeno sische Reaktorforschungs AG, Wuerenlingen) in 2 1 0.5 M phosphate buffer, pH 7.8. The reaction was carried

<sup>†</sup>Incubations contained 10 nmol of GalNAc-3H-G<sub>M2</sub> (16,000 c.p.m.), 50mM citrate-phosphate buffer (pH 4.4) and extract in a total volume of 200 µl. Incubations were for 3 h at 37° C and assay for GalNAc-3H released was as previously published<sup>3</sup>.

‡Incubations contained 10 nmol of GalNAc-3H-G<sub>A2</sub> (20,000 c.p.m.) 50 mM citrate phosphate buffer (pH 4.0), 0.24 mg sodium taurocholate.

and extract in a total volume of 200 µl. Incubations were for 1 h at 37° C and GalNAc-3H released was assayed as before.

out at room temperature for 20 h with constant stirring. A slight precipitate was removed by centrifugation; STH was then precipitated by the addition of 3 ml 1 M phosphate buffer, pH 7.0, prolactin by the addition of 2.2 ml saturated ammonium sulphate, pH 7.0, the precipitates collected and redissolved in 2 ml 0.05 M phosphate buffer, pH 7.8. This procedure was repeated four more times. The final supernatant contained negligible amounts of radioactivity. The precipitate was assumed to contain only hormone, since in preliminary trials the enzymes used did not precipitate in the conditions described. The protein concentration of the final solution was determined by optical density readings at 2800 Å, and the radioactivity of an aliquot determined. From this, a specific activity of  $5 \times 10^{16}$  d.p.m.  $mol^{-1}$  STH was calculated. Prolactin was labelled to the same extent.

Cell suspensions were prepared from calf thymus and brachial lymph nodes, obtained from the local slaughter-house within 15 min of killing the animal. Mouse thymus, mesenteric lymph nodes and thymus from mice injected 2 d previously with 2 mg of hydrocortisone were obtained from C3H/eb mice. The lymphoid organs were cut with scissors into fine pieces in cold Hank's balanced salt solution (BSS) and then squashed with a Teflon pestle against the walls of a loose fitting tube. Tissue debris was left to settle and the

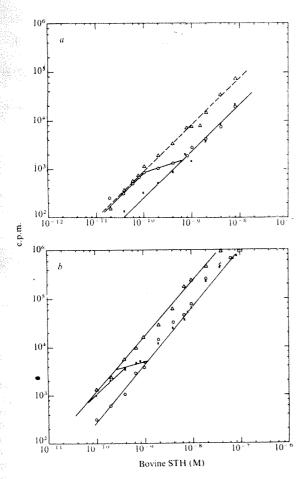


Fig. 1 a, Binding of bovine STH to bovine thymocytes (TC) and lymph node lymphocytes (LNC). Triplicate samples of  $2.5 \times 10^6$  cells were incubated in the cold for 15 min in 2 ml Hank's balanced salt solution containing labelled hormone at the indicated concentration. They were then spun down, 1 ml of the supernatant counted in a  $\gamma$  counter with an efficiency of 46%. Also, 1 ml of the hormone dilutions was counted to give the total c.p.m. added ( $\Delta$ ). The arithmetic mean of the differences between corresponding tubes containing cells and supernatant and only supernatant is given as c.p.m. bound to cells;  $\bigcirc$ , c.p.m. on bovine TC;  $\times$ , c.p.m. on bovine LNC. b, Binding of bovine STH to murine TC and LNC.  $\triangle$ , total c.p.m. added;  $\bigcirc$ , c.p.m. bound to mouse LNC;  $\times$ , c.p.m. bound to mouse TC.

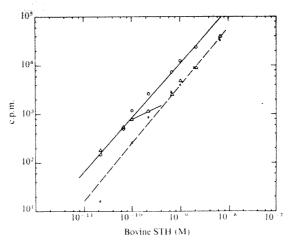


Fig. 2 Inhibition of binding of STH to murine thymocytes (TC) by pretreatment with an excess of STH or prolactin. Murine thymocytes were incubated in unlabelled hormone at a concentration of 10<sup>7</sup> cells per 10 μg hormone ml<sup>-1</sup> (15 min at 4°C) and washed twice with BSS. The rest of the procedure was as described in the legend to Fig. 1. Δ, c.p.m. on mouse TC, preincubated in bovine prolactin; (), total c.p.m. added; ×, c.p.m. on mouse TC, preincubated in bovine STH.

supernatant cell suspension was then washed once with cold BSS. Cell viability, as estimated by trypan blue exclusion, was over 90%. Cells were then incubated with decreasing amounts of labelled hormone in the cold and spun down. The radioactivity of half the supernatant was determined and separately that of the cell pellet with the other half of the supernatant. Differences in counts obtained were taken as evidence that hormone had bound to the cells and were used to estimate the amount of hormone bound by the cells (Fig. 1). It was found that bovine and murine lymph node cells (LNC) bound, at all the concentrations tested, about one third of the total amount of either STH (Fig. 1) or prolactin added (data not shown). Hydrocortisone-resistant murine thymocytes were similar to LNC in their binding behaviour. Normal thymocytes bound about the same fraction of the hormone at concentrations above 10-9 M; below that, however, they took up increasing proportions of the total STH present, but not of prolactin. At about 10<sup>-10</sup> M all the STH was associated with the cells. In contrast, prolactin was bound with decreasing efficiency by thymocytes at all concentrations below  $5 \times 10^{-10}$  M.

The binding of all the STH added to thymocytes at low concentrations could be reduced by about two-thirds by preincubating the cells in unlabelled STH (Fig. 2). Preincubation in unlabelled prolactin (Fig. 2) or thyroxin (2  $\mu$ g per  $10^{-7}$  cells in 2 ml BSS, 15 min at 4° C) had no effect upon the binding of STH to either LNC or thymocytes.

It therefore seems that there are two mechanisms by which STH binds to thymocytes: one demonstrable at high concentrations of the hormone, that is above 10° M, the other at physiological concentrations. The first one would account for the binding of about 1/3 of all the hormone added; it seems to be unspecific and not saturable, since binding could not be inhibited by preincubation of the cells in unlabelled STH or prolactin. This type of binding was also observed with lymph node lymphocytes and hydrocortisone resistant thymocytes. The other type of binding, observable at physiological concentrations of STH and below, seems to be saturable and specific, since it could be inhibited by preincubation of thymocytes in unlabelled STH but not of the structurally similar prolactin<sup>6</sup>. This binding is probably also of high affinity, since thymocytes incubated in labelled growth hormone (10<sup>7</sup> cells per 10 µg STH per ml, BSS, 15 min at 4° C) will, upon prolonged washing (12 centrifugations over a period of 24 h) retain an amount of

radioactivity per cell equivalent to that found at 10<sup>-9</sup> M STH in the dilution assay. In these conditions, lymph node cells shed all the radioactivity bound initially.

These data fit well with the assumption of specific receptors for STH being present in the thymocytes membrane.

The maximal amount of hormone taken up by the thymocytes through these postulated receptors can be estimated from Fig. 1, assuming saturation of the receptors at that concentration of hormone, when radioactivity first appears in the supernatant. From the specific activity of the hormone, the total number of cells in the incubation mixture, and the efficiency of counting, it may be calculated that about 10,000 molecules of STH were bound to one calf thymocyte and 20,000 to a murine thymocyte (average of three independent experiments, yielding, respectively, 18,000, 20,000 and 22,000). Whether this represents indeed the number of high affinity, specific binding sites on thymocytes is not clear as some of them may be occupied by endogenous hormone bound previously in vivo. The number of receptors may thus be substantially higher.

Specific binding of STH to cells has previously been demonstrated with human lymphocytes grown in vitro7 Whether the binding of STH to lymph node lymphocytes influences their physiological state is unknown. It is also unknown which part of the binding of STH to thymocytes induces changes in their metabolism in vitro and whether this is related to their biological activity2

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#### Initiation of polyphenylalanine synthesis and the action of cytokinins in fenugreek cotyledons

THE initiation of peptide chain formation is thought to be the point of control in protein synthesis. Plant growth hormones may also act at this point.

Isolated cotyledons of fenugreek (Trigonella foenum-graecum L.) respond to cytokinins1 and it has become evident that at an early stage the growth response in darkness is dependent on protein synthesis. It has been shown that cytokinin treatment of cotyledons increases the activity of the ribosome fraction in

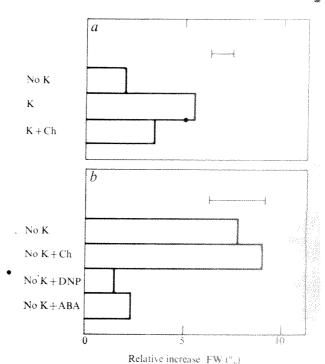


Fig. 1 Cotyledons (five per dish) were weighed before and after incubation at 25° C in darkness in Petri dishes (six replicates per treatment) on filter paper wetted with 20 mM KNO<sub>2</sub> and other compounds as indicated. Antibiotics used were penicillin, streptomycin and mycostatin, 25 µg ml<sup>-1</sup> each. LSD values at P=0.01 are shown. a, Stimulation of fresh weight increase of cotyledons after 6 h application of  $5\times10^{-6}$  M kinetin (K) and inhibition of this stimulation by 10  $\mu$ g ml<sup>-1</sup> cycloheximide (Ch), b, Inhibition of fresh weight increase after 47 h in the control (no kinetin) situation by  $5 \times 10^{-6}$  M dinitrophenol (DNP) and 5×10<sup>-7</sup>M abscisic acid (ABA) but not by 25 μg ml<sup>-3</sup> cycloheximide.

in vitro protein synthesis2. The ribosomes from treated coty ledons are more active in initiation of poly(U)-directed poly phenylalanine synthesis.

Cotyledonary petioles with enclosed plumules and radicle were removed from dry fenugreek seed by making two cuts The cotyledon containing parts were sterilised, washed, an incubated overnight in Petri dishes on moist filter paper. Th cotyledons were then isolated and subjected to a preliminar 24 h incubation in control conditions (20 mM KNO<sub>3</sub>, 50 µ ml-1 chloramphenicol) before such treatments were applied Other antibiotics were used, however, in some experiment (Fig. 1). Neither these antibiotics nor chloramphenicol affectes the growth response. From sterilisation onwards, the materia was in darkness except for manipulations carried out unde green safe light.

The growth response to cytokinins at its earliest observable stage seems to depend on protein synthesis. A stimulation of relative fresh weight increase (expansion) after 6 h of kineth application is shown in Fig. 1a; this stimulation was reduced significantly by cycloheximide at 10 µg ml<sup>-1</sup>. In contrast, ever after 47 h, the 'slow expansion' in the absence of cytokining was not inhibited by cycloheximide at 25 µg ml<sup>-1</sup>, (Fig. 1h) of a 10 μg ml<sup>-1</sup> (ref. 3). This is noteworthy because in the control condition, protein synthesis takes place and is inhibited by cycloheximide, and because the 'slow expansion' in the control condition is severely inhibited (Fig. 1b) by either dinitrophenol (5  $\times$  10<sup>-5</sup> M) or abscisic acid (5  $\times$  10<sup>-7</sup> M). Therefore cytokinin-induced growth in these cotyledons seems linked in a special manner to protein synthesis.

Significant effects on incorporation of amino acids have been observed 3, 2 and even 1 h after cytokinin application. In one experiment an increase of 48% of 14C-leucine incorporation into protein due to kinetin treatment was observed at the end of a 3 h incubation. In that experiment, cycloheximide (10 µ ml-1) inhibited incorporation in both the control and kinetir treatment to 16% of the uninhibited control value. Kinetin die

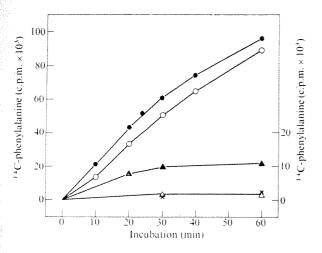


Fig. 2 <sup>14</sup>C-phenylalanine incorporation with time in an *in vitro* incorporation system without poly(U)(×), with poly(U)(○), and the same but after 5 min preincubation at 30° C of tRNA, poly(U) and ribosomes (♠). Aurintricarboxylic acid (5 μg) added at time zero (△) or after 5 min of preincubation on ice of tRNA, poly(U) and ribosomes (♠). Extracts were prepared by grinding lots of 100 cotyledons with mortar and pestle to a final volume of 12 ml of 0.1M Bis-Tris (bis (2-hydroxyethyl) amino tris (hydroxymethyl) methane) (Sigma) HCl, pH 7.8, 40 mM KCl, 10 mM MgCl<sub>2</sub>, 1 mM dithiothreitol. They were then centrifuged for 20 min at 18,000g and after addition of 200 μl 25% Triton X-100 the post-mitochondrial supernatants for 1 h at 160,000g. 5 ml of supernatant were dialysed for 2–3 h against 1 l of buffer; the 160,000g pellet was resuspended in buffer and centrifuged for 90 min at 160,000g. The pellet was again carefully resuspended, this time in about 2.5 ml of buffer and centrifuged once more at 5,000g for 5 min as was the dialysed supernatant. The absorbances at 260 nm were read and corrections made for differences by dilution. Assay: 30 min, 30° C, 0.5 ml total volume, buffer 0.1 M Bis-Tris pH 7.8, KCl 40 mM, MgCl<sub>2</sub> 10 mM, dithiothreitol mM, poly(U) 50 μg, tRNA (E. coli) 100 μg, ATP 1.2 μmol, GTP 0.1 μmol, PEP 1 μmol, pyruvate kinase in 4 M (NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub> (Sigma) 1 μl, ribosomes 2.5 A<sub>280</sub> units, supernatant 2.5 A<sub>280</sub> units. <sup>14</sup>C-phenylalanine 0.5 μCi, (522 mCi mmol<sup>-1</sup>). Stopped with TCA, heated and washed on to glass filter disks, digested with NCS solubiliser, and counted in toluene 2.5-diphenyloxazole scintillant mixture. \* No poly(U).

mot affect uptake but cycloheximide did; presumably this was a side effect of the inhibition of protein synthesis, as the inhibition of incorporation into protein was far in excess of that of uptake. In a repeat experiment, the 70% ethanol extractable amino acid content per cotyledon (4.7 s.e. 0.1 μmol) was not affected by the kinetin treatment.

In an earlier analysis using an *in vitro* protein synthesis system, it seemed that kinetin treatment of the cotyledons increased the activity of the ribosome fraction rather than that of the post ribosomal supernatant and its tRNA<sup>2</sup>.

My in vitro amino acid incorporating system used vashed, Triton-treated ribosome and dialysed high-speed supernatant fractions. The supernatant used throughout was derived from control cotyledons but the ribosomes were from either control or cytokinin-treated cotyledons and corrected or differences in concentration (see legend to Fig. 2).

Incorporation in the poly(U)-directed assay system proceeds inearly with time for about 30 min, or it may show an initial ag; but, if poly(U), ribosomes and tRNA are preincubated in buffer together, the initial lag in incorporation is removed when the other components of the assay system are added Fig. 2). This suggests the requirement for an initiation step in polyphenylalanine synthesis, in which a ribosome-poly(U) complex is formed<sup>4</sup>. The initiation process may be stopped nearly completely and specifically in plant systems<sup>5</sup> at any time by addition of  $2 \times 10^{-5}$  M aurintricarboxylic acid (ATA); applied at zero time it removes the effects of poly(U) addition (Fig. 2). Although a preincubation, carried out at  $0^{\circ}$  C, did not seem to semove the initial lag, such preincubation does seem to afford

Table 1 Effect of treatment of cotyledons with various cytokinins for 3 h (experiment 1) with kinetin and inhibitors (experiment 2) on initiation activity

	<sup>14</sup> C-Phenylalanine Incorporation (c.p.m.) after 30 min					
75				30 min		
Treatment of cotyledons		preincu				
	With	Without	Difference	Relative value		
Experiment 1 (3 h)				varue		
Control	4,555	884	3,671	100		
Kinetin 5×10 <sup>-5</sup> M	9,291	973	8.318	227		
Zeatin 1×10 <sup>-6</sup> M	12,029	992	11,037	301		
6-Benzyladenine 5×10 <sup>-6</sup>		933	6,610	180		
6-Methyladenine 5 × 10 <sup>-5</sup>		671	3,089	84		
Experiment 2						
Control	5,645	1,550	4,095	100		
Kinetin 5 × 10 <sup>-5</sup> M, 3 h	10,203	1,707	8,496	207		
2, 4 Dinitrophenol	,	2,.07	0,150	20.		
$(1 \times 10^{-4} \text{ M}), 20 \text{ h}$	4,373	862	3,511	86		
2,4 Dinitrophenol	1,2		0,0			
$(1 \times 10^{-4} \text{ M}), 20 \text{ h}$						
+ kinetin ( $5 \times 10^{-5}$ M),	3 h 3,916	923	2,993	73		

Activity expressed as difference in incorporation of <sup>14</sup>C-phenylalanine after 30 min incubation of a poly (U)-directed system with €×10<sup>-5</sup> M aurintricarboxylic acid added at time zero but with and without a preincubation of poly(U), tRNA and ribosomes for 5 min on ice as in Figs 2 and 3. Control supernatant was used in all incubations.

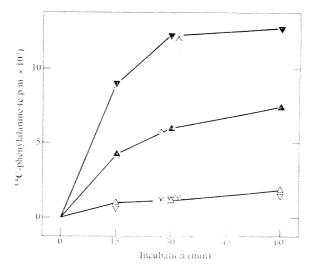
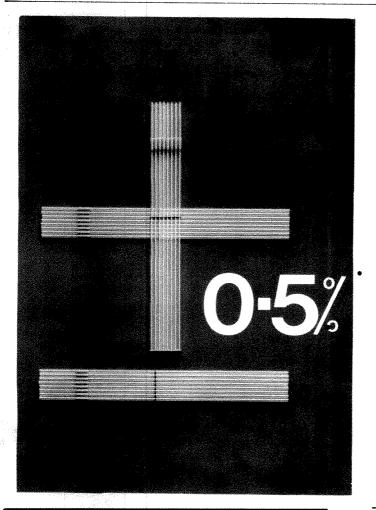


Fig. 3 Measurement of initiation activity of ribosomes from control ( $\triangle$ ) and  $5 \times 10^{-5}$  M, 3 h, kinetin-treated ( $\nabla$ ) cotyledons as difference (arrows) in incorporation of <sup>14</sup>C-phenylalanine in a poly(U)-directed system with aurintricarboxylic acid added at time zero but with ( $\triangle$ ) and without ( $\triangle$ ) 5 min preincubation on ice of tRNA, poly(U) and ribosomes, as in Fig. 2, but during incubation only control supernatant used.

measurable initiation activity: ATA, applied after a 5 min initiation preincubation of poly(U), ribosomes and tRNA on ice, allows some extra incorporation during the subsequent elongation—incubation period (Fig. 2).

A measure of the initiation activity is obtained by the difference between incorporations in incubations which had or had not followed preincubation of ribosomes, poly(U) and tRNA for 5 min on ice and to which ATA had been added at time zero (Fig. 3). Table 1 shows the relative values in initiation activity using ribosomes from cotyledons treated in different ways. The activity is increased considerably after treatment for 3 h with kinetin, zeatin or 6-benzyladenine, but notably not by 6-methyladenine, a base which at the applied concentration does not elicit a growth response<sup>1</sup>.

No increase in initiation activity, however, has been observed following *in vitro* treatment of control ribosomes with zeatin ( $10^{-6}$  M) or with 6-benzyladenine ( $5 \times 10^{-6}$  M). The increase in activity of ribosomes from kinetin treated cotyledons is pre-



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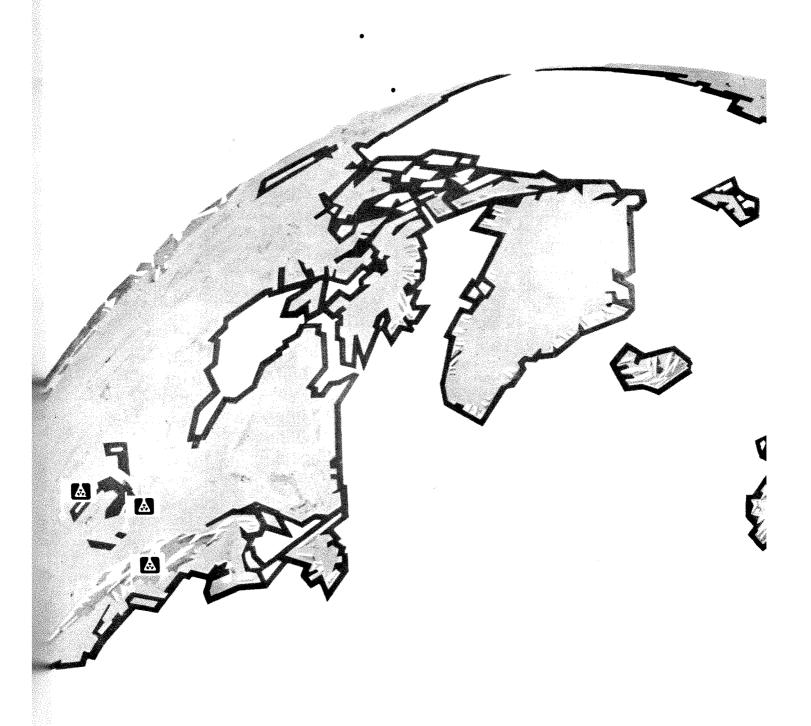
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vented by the presence of 2,4-dinitrophenol for 20 h during the treatment of the cotyledons receiving kinetin during the last 3 h only (Table 1). This suggests that the kinetin effect on initiation activity requires energy.

For mammalian ribosomes, binding of poly(U) to the small subunit at 0° C has been shown to be dependent on the presence of associated protein. For this protein, which is removed by a 0.5 M KCl wash, initiation-factor function has been suggested. In several experiments, not detailed here, the presence of 0.5 M KCl in the buffer used for resuspending the ribosomes after the first high speed centrifugation, about halved the initiation activity of the ribosomes, which after the second high speed centrifugation were resuspended in standard buffer. A way in which the present kinetin-induced increases in initiation activity may be explained is as an increase in the concentration or as an activation of protein initiation factors.

My findings may help to explain the function of the reported binding of cytokinins to ribosomes7. Such binding by itself, however, does not seem sufficient to explain the observed increase in initiation activity. An improvement of the ribosome fraction has been observed for auxin treated soybean hypocotyl segments and it was suggested that this implied an enhanced level of peptide chain initiation<sup>8</sup>.

I thank Mrs L. Eckhardt for technical assistance and Dr J. A. Zwar for correcting the text.

Addendum: In the experiment of Fig. 2 the ribosomes were prepared from freshly dissected cotyledons as such ribosomes seem to show the lag period more readily.

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#### A receptor mediating sexual differentiation?

SEXUAL differentiation of the brain is believed to be mediated by exposure to testicular androgens at a critical period of development-prenatal in most species, but during the first 5 d after birth in rats. Only those androgens which are potential substrates for aromatising enzymes (that is, those which can be converted to oestrogens in vivo) are effective1-4. These observations led to the concept that masculinisation of the brain is caused by local conversion of androgens to oestrogens.

There is now a considerable amount of evidence in support of this hypothesis. For example, the masculinising effects of androgens on the brain can be prevented by the previous administration of the antioestrogen MER 25 (ref. 5), and the effects of oestrogens, administered during the critical period, are similar to those of androgens<sup>6-9</sup>. Aromatising enzymes are present in the hypothalamus and amygdala<sup>10</sup> and in vivo conversion of 3H-androgen to 3H-oestrogen has been shown

in these areas during the critical period11.

Are oestrogen receptors present in the brain during the critical period? Previous attempts12 to answer this question have been hampered by the presence of large amounts of a specific oestrogen-binding protein (blood EBP)13 in the plasma of perinatal animals. Neonatal rat brain contains a similar protein (brain EBP)14 which is present in high concentrations and is possibly extracellular. It is distinguished from the oestrogen receptor of the adult brain15 by its relatively low affinity  $(K_d \sim 10^{-8} \text{ M})$  for oestradiol and by the inability of diethyl-stilboestrol (DES) to compete with oestradiol-17β in the binding reaction.

Here we report the use of a new and sensitive method<sup>15</sup> to estimate the high affinity binding of oestradiol by cytosol from the brains of neonatal rats. We chose our experimental conditions so that we measured only slowly dissociating, high affinity, DES suppressible binding; the low affinity EBPs of blood and brain were not detectable.

Wistar rats (5 d old) of both sexes were used and binding was estimated in cytosols prepared from two areas of the brain: (HA) which contained both hypothalamus and amygdala, and (C) which contained cerebral cortex. Scatchard<sup>17</sup> representations of binding isotherms (Fig. 1) were apparently rectilinear: this is consistent with the measurement of a single class of noninteracting binding sites. Dissociation constants of the reaction were similar to those of the adult receptor, about 10<sup>-10</sup> M (Table 1).

The distribution, however, was different. In the adult the

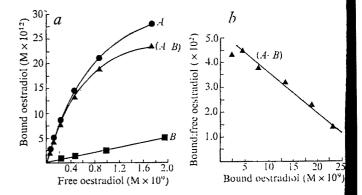


Fig. 1 a, The binding of <sup>3</sup>H-oestradiol to cytosol from brain of 5-d-old rats; cytosol was from a female in this example. Cytosol (110,000 $g \times 1$  h; phosphate buffer, pH 7.3) samples Cytosoi (110,000 $g \times 1$  n; phosphate duffer, p = 7.3) samples were incubated with various concentrations (2 × 10<sup>-9</sup> M to 2 × 10<sup>-10</sup> M) of 2, 4, 6, 7 <sup>3</sup>H-oestradiol-17g (100 Ci mmol<sup>-1</sup>) for 30 min at 30° C. Control incubations contained, in addition, an excess, (10<sup>-7</sup> M) of unlabelled DES. 0.2 ml fractions were placed on small columns  $(0.45 \times 10 \text{ cm})$  of Sephadex LH-20 in buffer, maintained at  $0-4^{\circ}$  C. Samples were washed with 0.2 ml buffer and allowed to remain in contact with the gel for 150 min before they were eluted (0.7 ml), and bound radio-activity estimated. A, total binding; B, binding in the presence of excess DES; (A-B) DES suppressible binding, obtained by subtraction. b, Scatchard representation of A-B. We detected no DES suppressible binding in either blood or heart cytosol.

receptors are most concentrated in hypothalamus and amygdala and are relatively sparse in cortex. In the brain of the neonatal animal equal amounts of high affinity oestrogen binding material were found in both HA and C and in both sexes (Table 1); concentrations were similar to those of the receptor in the adult female hypothalamus. The binding material is, a least in part, protein: incubation with pronase, but not with DNase or RNase, destroyed its ability to bind oestradiol Binding activity was reduced after incubation with protamine sulphate or with p-hydroxymercuribenzoate. Thus the protein is similar to the adult receptor in that it is acidic, and the

Table 1 Distribution and dissociation constants of reaction (K<sub>d</sub>) of DES suppressible, high affinity binding of <sup>3</sup>H- oestradiol-17β, in cytosol from brain of 5-d-old rats

	Abundance of oestradiol	binding sites	Reaction dissociation constant			
	Sites $g^{-1}$ wet weight ( $\times 10^{-10}$ )		$K_{\rm d}   imes  10^{10}   ({ m M})$			
	Hypothalamus Amygdala	Cortex	Hypothalamus Amygdala	Cortex		
Adult female	$19.8 \pm 1.2$ $8.3 \pm 0.4$	$1.8 \pm 0.3$	$1.5 \pm 0.3$ $1.08 \pm 0.16$	$1.7 \pm 0.6$		
5-d-old female	$11.8 \pm 1.8$	$13.4 \pm 1.1$	$6.1 \pm 1.2$	$6.4 \pm 1.5$		
5-d-old male	$10.7 \pm 1.0$	$15.9 \pm 1.9$	$5.7 \pm 0.6$	$6.3 \pm 1.0$		

Data are compared with values for binding in cytosols from brain of adult female rats<sup>15</sup>. Parameters were calculated from Scatchard representation of binding isotherms. Avogadro's number was used in the calculation of the number of binding sites. Values represent means  $\pm$  s.e.m. of at least five observations.

Table 2 The specificity of the binding reaction in cytosol from brains of 5-d-old rats compared with that of the receptor of the adult female

		· Adult		5-d-old			
		Order of	<u>ұ</u> <b>Н</b>	9	2		3 <sup>*</sup> _
Competitor		$K_{\mathrm{d}}$	H	HA	С	HA	C
Ethamoxytriphetol	-(MER 25)	10−6 M	$\sim 1$	<b>∼</b> 1	<b>∼</b> 1	<b>∼</b> 1	<b>∼</b> 1
5α-Androstan-3α, 17β-dio		10⁻⁻¹ M	$3.4 \pm 0.6$	$4.8 \pm 1.3$	$5.9 \pm 2.3$	$3.4 \pm 1.2$	<b>∼</b> 10
5α-Androstan-3β, 17β-dio	—(3β diol)	10−8 M	$3.3 \pm 0.4$	$6.4 \pm 1.9$	$9.1 \pm 3.4$	$7.9 \pm 1.1$	$6.0 \pm 2.6$
trans Clomiphene	—(TCl)	10 <sup>-8</sup> M	$8.0 \pm 1.5$	$11.4 \pm 6.1$	$10.1 \pm 3.3$	$14.4 \pm 3.7$	$13.4 \pm 2.5$
cis Clomiphene	—(cis Ćl)	10−8 M	$1.3 \pm 0.5$	$0.62 \pm 0.22$	4.6	$4.1 \pm 2.4$	$1.0 \pm 0.17$
Oestriol -	$-(E_3)$	10 −9 M	$1.7 \pm 0.1$	$1.2 \pm 0.5$	$2.5 \pm 1.5$	$2.8 \pm 1.0$	$5.3 \pm 3.6$
17α-Oestradiol	$-(17\alpha)$	10 −9 M	$2.2 \pm 0.2$	$3.4 \pm 1.4$	$3.2 \pm 0.8$	$5.5 \pm 2.3$	$3.7 \pm 1.7$
Oestrone	$-(E_1)$	10 <sup>-9</sup> M	$1.7 \pm 0.1$	$31 \pm 7$	22 土 4	$12 \pm 4$	$29 \pm 14$
16-Epi oestriol	—(Epi) E₃	10 <sup>−9</sup> M	$1.3 \pm 0.1$	$2.0 \pm 0.6$	$3.9 \pm 1.7$	$0.8 \pm 0.2$	$3.4 \pm 1.9$
11β-Methoxy-17α-ethynyl-	( T )						
17β-oestradiol	(Ru 2858)	10 <sup>-10</sup> M	$7.1 \pm 0.7$	$6.0 \pm 1.5$	$4.1 \pm 1.1$	$5.2 \pm 1.6$	$5.6 \pm 1.4$
	$-(17\beta)$	10 <sup>-10</sup> M	$1.2 \pm 0.1$	$5.7 \pm 1.7$	$5.5 \pm 1.0$	$3.2 \pm 0.6$	$3.7 \pm 1.1$
DES		10 <sup>-11</sup> M	$6.0\pm1.0$	$8.5 \pm 0.9$	$11.3 \pm 3.1$	$9.8 \pm 0.3$	$6.4 \pm 1.1$

Dissociation constants of reaction were calculated from the suppression in the presence of unlabelled test substances of binding of 3H-oestradiol in the conditions specified in the legend to Fig. 1. Where standard errors are quoted the data are the mean values of four observations. Testosterone, 5α-dihydrotestosterone, progesterone, corticosterone and cyproterone (10-6 M) had no effect on the binding of <sup>3</sup>H-oestradiol.

integrity of -SH groups is important for the binding reaction.

The specificity of the reaction is the same in both areas and in both sexes and is broadly similar to the adult receptor (Table 2): potent oestrogens have high, and non-oestrogenic steroids have low binding affinities.

The low affinity for MER 25 of both the oestradiol receptor of adult hypothalamus and the oestrogen binding material from neonatal brain is somewhat surprising; however, MER 25 is an effective antioestrogen only when administered in high doses<sup>16</sup> and it protects the brain against masculinisation only when given some time before exposure to androgen<sup>5</sup>.

The affinities of the protein in brain cytosol from 5-d-old rats for oestradiol-17β and for oestrone are apparently lower than those of the adult (Table 2): but these differences may be artefacts due to the presence of brain EBP in the incubation medium.

The high binding affinities of the synthetic oestrogens DES and Ru 2858 are particularly interesting. Both compounds have a low affinity for blood EBP (ref. 13) and both affect the developing brain in a manner similar to that of oestradiol. Ru 2858, at least, is very much more potent than oestradiol-17β in its effects on the developing brain (C. Doughty, unpublished).

We propose that the high affinity, oestrogen-specific binding protein of neonatal brain cytosol is a receptor which is involved in the sexual differentiation of the central nervous system. Recent autoradiographic work<sup>12</sup> supports this view. After injection of 3H-oestradiol into newborn female rats radioactivity was concentrated in the nuclei of cells in the hypothalamus and amygdala: uptake was inhibited by concurrent administration of oestradiol or testosterone, but not by unlabelled 5α-dihydrotestosterone, which cannot be converted to oestrogen in vivo.

The role of the putative cytoplasmic receptor of the neonatal cortex has yet to be determined. As aromatising enzymes are absent in the cortex it is unlikely to be involved in the normal

process of masculinisation but may be involved in a, so far undefined, organising effect of oestradiol itself.

This work was supported by the Medical Research Council.

JACKIE BARLEY M. GINSBURG B. D. GREENSTEIN N. J. MACLUSKY P. J. THOMAS

Chelsea College, University of London, London, UK

Received August 2, 1974.

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#### APPOINTMENTS VACANT

#### THE FACULTY OF MEDICINE OF MEMORIAL UNIVERSITY OF NEWFOUNDLAND

will appoint an Endocrinologist to its Basic Sciences Division in 1975. Individuals with research in-terests in metabolic regulation, neuroendocrinology or membrane studies will be considered.

Interested applicants should send resumé and names of three referces to:

Dr. Bruce H. Sells
Laboratories of Molecular Biology
Faculty of Medicine
Memorial University of Newfoundland
St. John's, Newfoundland

Before December 15, 1974.

(1755)

#### UNIVERSITY OF ALBERTA DEPARTMENT OF ZOOLOGY Edmonton, Alberta T6G 2E1, Canada

Edmonton, Alberta T6G 2EI, Canada

Two positions are available as Assistant or Associate Professor, from July 1, 1975. A specialist in some aspect of invertebrate zoology is needed to take major responsibility for teaching in a general course in invertebrates. A specialist in vertebrate morphology and vertebrate paleontology; an interest in fishes is preferred. In both positions there will be opportunity for teaching at advanced levels. Strong research orientation and Ph.D., or equivalent, is required. Salary will be within the range Can \$13,440 to 23,416. Applicants send a curriculum vitae and the names of three referees to Dr. J. R. Nursall, Chairman, Department of Zoology, before January 31, 1975. (1756)

#### THE HORMEL INSTITUTE OF THE UNIVERSITY OF MINNESOTA invites applications from qualled individuals for the position of

#### EXECUTIVE DIRECTOR

EXECUTIVE DIRECTOR

of the Hormel Institute which is located in Austin, Minnesota. The Executive Director is responsible for overall administration of an institute with 80-90 employees, including 20 of whom have academic rank. Candidates for this position should be recognized authorities in the lipid field, and should have at least 12 years of research experience in the field. The director is expected to maintain an active research program. Closing date for receipt of all materials is February 1, 1975. Send Curriculum vitae, a list of publications and names of three referees to Dr. J. E. Gander (Chairman, Search Committee), Department of Biochemistry, University of Minnesota, St. Paul, MN 55108. (1757)

#### UNIVERSITY OF BIRMINGHAM TECHNICIAN GRADE 3

A Research Technician is required to assist in a Neurochemistry laboratory, in the Neurocommunications Research Unit. The work involved would mainly be biochemical with some physiological and animal experimentation. Some laboratory experience essential and preference will be given to applicants with a degree or O.N.C. in Chemistry or a Biological subject.

Apply:

Assistant Secretary, University of Birmingham, P.O. Box 363, Birmingham B15 2TT.

(1763)

Ref. 581/C/579.

## **International Consultants**

## Tropical Agriculture

Bookers Agricultural and Technical Services manage sugar estates and provide a wide range of consultancy services for sugar and other agro-industrial projects. Assignments have been undertaken in over thirty-five countries on behalf of the World Bank, UN Agencies, ODM, overseas governments and

Further growth has created career opportunities for qualified AGRICULTURALISTS and AGRICULTURAL ENGINEERS who can make a significant contribution to agricultural expansion in developing countries. The work, which involves both agricultural planning and large-scale project development, requires frequent travel, with periods of overseas residence as

Candidates, ideally 32-45, should have a minimum of ten years' broadly-based agricultural experience, of which not less than five years should have been spent in non-temperate regions. A first-hand knowledge of the sugar industry would be valuable.

The appointments carry attractive salaries, overseas and travel allowances as appropriate, and substantial fringe benefits.

Please send brief career and personal details in the first instance to:

E. C. Robinson, Personnel Director, Bookers Agricultural and Technical Services Limited, Bucklersbury House, 83 Cannon Street, London EC4N 8EJ.



(1851)

## **Basic Grade Biochemist**

Required in the Biochemistry Department at the Royal Infirmary. The department is well equipped with a range of automated and manual instrumentation. It serves approximately 3,000 beds, including specialist units, and all General Practitioners. There are five other graduate staff. Previous experience is desirable but not essential.

The candidate appointed will be required to undertake studies leading to a professional or academic higher degree. Attendance at scientific meetings of the Association of Clinical Biochemists and at courses leading to further qualifications will be encouraged. Whitley Council salary scale and conditions of service. Removal expenses where appropriate can be paid.

Applications to Personnel Officer, Victoria House, Park Street, Hull.

Further enquiries and for engagements to visit the Laboratory, contact Dr L. B. Roberts, 0482-28541, Ext.



#### **Hull District**

Humberside Area Health Authority

(1852)

## STATE OF KUWAIT KUWAIT UNIVERSITY

#### ACADEMIC POSTS FOR 1975-1976

Applications are invited for the posts of Lecturers, Assistant Professors, and Professors. Contracts start on September 1, 1975, for two years. Renewable for a further period of four years if convenient to both applicant and university.

Applicants should be:-

- (A) Holders of an academic post at present in an accredited university or research center.
- (B) Ph.D. holders.

Application and curriculum vitae form with particulars of salary and other matters, are obtainable from Kuwait Embassies in Washington D.C. (4301) Connecticut Avenue, N.W., Site 158, Washington D.C. 200083 and London Cultural Attache Office, Al-Jahra House, 3 Stratford Place, London WIN 9AE, or from Kuwait University, Kuwait. Completed applications, together with a copy of the candidate's publications, must be received by 'Kuwait University, Kuwait', not later than December 15, 1974. Those who applied last year and this year can renew their applications by writing to the university.

Candidate is also entitled to the following privileges:

- 1. Annual return air tickets to this country, would be provided to him, his wife and three of his children not exceeding the age of twenty.
- 2. Free furnished accommodation with water and electric

A-FACULTY OF SCIENCE:-

- MATHEMATICS:
  (1) APPLIED MATH
  (2) PURE MATH
  (3) COMPUTER SCIENCE

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  (1) SOLID STATE PHYSICS

  (2) ATOMIC PHYSICS

  (3) HIGH ENERGY PHYSICS

  PARTICLES PHYSICS-ELEMENTARY

- PARTICLES

  EXPERIMENTAL PHYSICS:

  (4) ATOMIC PHYSICS

  (5) LASER PHYSICS

  (6) ATMOSPHERIC PHYSICS

  (7) GEOPHYSICS-ASTROPHYSICS

  (8) ELECTRONICS

  (9) EXPERIMENTAL PHYSICIST (with a good experience in setting up and developing undergraduate physics laboratories in addition to a research interest in one field of experimental physics.)

  CHEMISTRY:

- CHEMISTRY:

  (1) BIOCHEMISTRY

  (2) INORGANIC CHEMISTRY

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  (4) ORGANIC CHEMISTRY

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  (6) PHYTOCHEMISTRY
  (7) PHYSIOLOGICAL ECOLOGY

- (7) PHYSIOLOGICAL ECOLOGY

  ZOOLOGY:

  (1) MARINE ICHTHYOLOGY

  (2) MARINE PHYSIOLOGY

  (3) MARINE ECOLOGY

  (4) MARINE FISHERIES AND MARICULTURE

  (5) OCEANOGRAPHY

  (6) IMMUNOLOGY

  (7) HISTOLOGY AND HISTOCHEMISTRY

  (8) MARINE FARASITOLOGY

- (8) MARKING
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  (1) ENVIRONMENTAL GEOLOGY
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  (4) GEOHYDROLOGY
  MARINE GEOLOGY

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  (2) ENGLISH LITERATURE-DRAMA-(ELIZA-BETHAN AND RESTORATION PERIOD)

  (3) THE NOVEL (20TH CENTURY)

  (4) HISTORY OF CIVILISATION

  (5) THE NOVEL (19TH CENTURY)

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  AND LOGIC
  (3) ANCIENT ORIENTAL PHILOSOPHY
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  (2) GENERAL PSYCHOLOGY

  (3) SOCIAL PSYCHOLOGY

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    FOUNDATIONS OF EDUCATION
    CURRICULUM AND TEACHING METHOD
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C-FACULTY OF LAW AND SHARI'A:-SHARI'A AND ISLAMIC STUDIES:

(1) PRINCIPLE OF MOSLEM FIKH
PRIVATE LAW:

(1) CIVIL LAW

(2) LAW OF PROCEDURE

PUBLIC LAW:
(1) PUBLIC FINANCE
(2) PUBLIC LAW

INTERNATIONAL LAW:
(1) PUBLIC INTERNATIONAL LAW

- PENAL LAW:
  (1) CRIMINAL LAW
- D—FACULTY OF COMMERCE, ECONOMICS AND POLITICAL SCIENCE:—

  ACCOUNTING AND AUDITING DEPARTMENT:

  (1) FINANCIAL ACCOUNTING
  (2) MANAGERIAL ACCOUNTING
  (3) PETROLEUM ACCOUNTING

  BUSINESS ADMINISTRATION:

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  ECONOMICS:

**ECONOMICS** 

ONOMICS:

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(2) ECONOMIC OF PRODUCTION

(3) ECONOMIC OF INDUSTRY AND TRANS-PORTATION

(4) LABOUR ECONOMICS

(5) PUBLIC FINANCE

(6) URBAN ECONOMICS

LITICAL SCIENCE.

POLITICAL SCIENCE:

(1) PUBLIC ADMINISTRATION

(2) POLITICAL SCIENCE WITH SPECIALIZATION IN POLITICAL BEHAVIOUR AND METHODS OF SCIENTIFIC RESEARCH

STATISTICS:
(1) STATISTICS
(2) INSURANCE

#### UNIVERSITY OF GUELPH DEPARTMENT OF CHEMISTRY **FACULTY POSITION**

Applications are invited for a faculty position in the area of biochemistry or biophysical chemistry at the rank of assistant or associate professor, to begin September 1, 1975. Candidates must have a Ph.D. degree in chemistry or biochemistry and at least one year of postdoctoral experience in biochemistry or biophysical chemistry. Duties will include undergraduate and graduate teaching in biochemistry, supervision of graduate research, and independent research. Salary and rank will depend on qualifications and experience.

Applicants should provide a full curriculum vitae, transcripts of academic record, a brief description of research interests and a research proposal, and the names of three referees to A. K. Colter, Chairman, Department of Chemistry, University of Guelph, Guelph, Ontario, Canada, N1G 2WI. (1750)

#### ACADEMIC OPENING

The Department of Chemistry of Yale University is seeking applications for appointment to our faculty at the Assistant Professor level in inorganic chemistry. The appointment would become effective in the 1975-76 academic year. Expertise in x-ray crystallography is required; however, a research program in structural chemistry should complement a program in another subdiscipline, such as bioinorganic or organometallic chemistry.

Yale has an active Affirmative Action Program and we are especially interested in minority group or female candidates who meet the above criteria.

or female candidates who meet the above criteria.

Candidates should request referees to write and should send a curriculum vitae, a list of publications, brief summaries of predoctoral and any postdoctoral research, and a brief outline of proposed research projects to the Chairman, Department of Chemistry, Yale University, New Haven, Connecticut, 06520. Applications should be sent promptly, preferably by December 31, 1974 to assure consideration. (1773)

#### **IMPERIAL** COLLEGE

Applications are invited for a POSTDOCTORAL RESEARCH AS-SISTANT or POSTGRADUATE STUDENT, to develop light scattering techniques for determination of the size and refractive index of particles in flames. Applicants should have good qualifications, or have adequate experience, in light scattering and related fields.

Applications, including curricula vitae and the names of two referees, should be sent to Dr A. R. Jones, Department of Chemical Engineering and Chemical Technology, Imperial College, London SW7 2BY.

(1885)

#### UNIVERSITY OF BRISTOL DEPARTMENT OF BIOCHEMISTRY POSTDOCTORAL RESEARCH **ASSISTANTSHIP**

Applications are invited from candidates with research experience in protein chemistry and/or enzymology for a Postdoctoral Research Assistantship to work in collaboration with Dr J. Williams on an M.R.C. supported project involving a structural and functional investigation of metal-binding fragments from transferrin.

The appointment is available now for a period of up to three years at an initial salary up to £2,412 (plus benefit of Threshold Agreement supplement as appropriate).

Interested candidates should write to: Dr J. Williams, Molecular Enzymology Laboratory, Department of Biochemistry, University of Bristol, Medical School, Bristol BSS 1TD. (1784)

## **Analyst For Drug Metabolism**

Applications for this post are invited from scientists with experience of analytical methods and instrumentation applicable to Drug Metabolism studies. They should be able to develop new analytical methods, should be familiar with the techniques of spectrophotometry, fluorimetry, all types of chromatography and mass spectrometry, and should be able to subject their results to kinetic analysis. Preference will be given to candidates with some knowledge of the metabolic processess affecting non-endogenous compounds.

We are seeking someone with research experience but we will consider new graduates who have taken relevant degree courses.

We encourage publication of appropriate work. We have active links with a number of university departments, units of clinical pharmacology and physicians involved in drug trials.

Working conditions include a good career/salary structure, flexible working hours and a basic annual holiday of four weeks. Various attractive residential areas are within easy reach of the Research Institute and help may be provided with removal expenses where appropriate.

Please apply in writing with brief details of experience, salary etc., to the Personnel Manager, May & Baker Ltd., Dagenham, Essex or telephone for an application form to Mrs A. E. Jones on 01-592 3060 Extension 506. To help us please quote reference No. 260/N/1.



(1859)

#### **BOTSWANA**

## FISHERIES SUPERINTENDENT

Required to supervise staff in the Department of Agriculture engaged on the manufacture and maintenance of fisheries equipment, and to design special equipment for use in the Fisheries Section. He will also be involved on some development project work.

Candidates should have a Diploma in Fishery with at least 5 years' appropriate experience.

Salary will be in the scale £2,308 to £3,710 p.a. which includes an allowance, normally tax-free, currently in the scale £510 to £1,146. This allowance is under review, and a new scale ranging from £654 to £1,902 is expected to be approved shortly. Initial tour of two to three years.

Benefits include: 25% gratuity on basic salary, paid passage, education allowances, holiday visit passages, generous leave, accommodation at reasonable rental. A car loan up to £600 and an appointment grant up to £300 may also be payable.

The post described is partly financed by Britain's programme of aid to the developing countries administered by the Ministry of Overseas Development.

For further particulars you should apply, giving brief details of experience to:

## crown age

M Division, 4 Millbank, London SW1P 3JD, quoting reference number MID/740816/NF (1876)

#### **BIOCHEMICAL TECHNICIAN**

Biochemical Technician required for small laboratory engaged in Metabolic Studies. Some experience with enzymes an advantage. Salary £1,800 to £2,800. Please apply with details of experience and names and addresses of two referees to: The Director, Alfred Chester Beatty Body Dynamics Laboratory, Bro Cranbrook, Kent TN17 3DT. Brooksden,

(1897)

#### UNIVERSITY OF MANCHESTER SENIOR LECTURER IN EXPERIMENTAL PATHOLOGY

EXPERIMENTAL PATHOLOGY

Applications are invited for this post in the Department of Pathology. The post involves teaching the principles of Pathology and research within a group investigating the immunological and migratory properties of lymphocytes. This group which is partly supported by an M.R.C. programme is concerned with the recognition structures on lymphocytes and endothelial cells which govern the migration of lymphocytes from the blood into the tissues. For this project experience of membrane fractionation techniques or glycoprotein chemistry would be relevant. A second project is concerned with the recognition of transplantation and other antigens by T cells. The post will not carry clinical duties. Salary range p.a.: £4,707 to £5,844. F.S.S.U. Further particulars and application forms (returnable by December 21) from the Registrar, The University, Manchester M13 9PL. Quote Ref. 229/74/N. (1809)

#### UNIVERSITY OF MANITOBA FACULTY OF MEDICINE

invites applications and nominations position of for the

#### PROFESSOR AND HEAD

#### DEPARTMENT OF BIOCHEMISTRY

DEPARTMENT OF BIOCHEMISTRY

Candidates should have a substantial record of accomplishment in Biochemical research as well as extensive teaching experience. The Department is responsible for the teaching of Biochemistry to medical students and also has an established graduate program leading to the Ph.D. degree. Applications and nominations should be accompanied by a curriculum vitae and submitted to:

Dr. Henry G. Friesen
Chairman: Biochemistry Search Committee
Department of Physiology
Faculty of Medicine
University of Manitoba
Winnipeg, Manitoba
R3E OW3, Canada (1861)

## Papua New Guinea

## Land Utilisation Officers

Applications are invited from graduates in agricultural science or science with a major in soil science for posts with the Department of Agriculture, Stock and Fisheries. Candidates should have good postgraduate experience and proven abilities detailed in either a) or b) below:-

a) Class 2. Experience in the conduct and successful completion of soil and land use surveys is essential. Appointees will be involved in reconnaissance surveys in the field or the collection and collation of data with

investigations into specialised fields of pedology, land use, soil physics and conservation.

b) Class 3. Working experience as a Pedologist with managerial and interpretative responsibilities in applied land use essential. Appointment will be made either to control a major regional soil survey unit and originate programmes for laboratory and field study or to be involved in reviving land utilisation patterns against a background of economic and technological advances.

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Period of engagement is for two years (renewable in most instances). General entitlements are very attractive and include a

generous gratuity (approx. 25% of salary combined with attraction allowance), education allowance for dependent children attending school overseas, return air passages with personal effects and luggage allowance, low cost married and single accommodation, and generous leave conditions.

Please write or telephone immediately for an application form and full details of the posts. The Papua New Guinea Public Service Board Representative, 22 Garrick Street, London W.C.2. Telephone: 01-240 1780.

Papua New Guinea



#### DIRECTOR

Applications are invited for the position of DIREKTOR DES INSTITUTS FÜR TIERZUCHT UND TIERVERHALTEN FORSCHUNGSANSTALT FÜR LANDWIRTSCHAFT,

a federal research institution engaged in reproductive biology, animal behaviour and genetics. Staff of 250 including 16 scientists. Research budgeted, Facilities: 2,500 m<sup>2</sup> of laboratory space; 4 research farms (1200 ha); live stock of 1,200 cattle, 2,000 pigs, 1,000 sheep. Locations: main laboratories and 2 farms at Mariensee near Hannover; laboratory and 2 farms at Trenthorst near Lübeck.

Qualifications: Internationally established research reputation. Applicants with biochemical and physiological background should be enabled to coordinate the research programmes of the different restrons.

Salary: approx. 65,000 DM/annum (B 3 tenure position in government service).

To apply, send curriculum vitae, summary of research activities, list of publications and reprints before December 31, 1975 to:
Prof. Dr E. Zimmer
Präsident of
Forschungsanstalt für Landwirtschaft
Bundesaltes 50

Bundesallee 50 D 33 Braunschweig Federal Republic of Germany

(1792)

#### NATIONAL COUNCIL FOR SCIENTIFIC RESEARCH

Applications are invited from suitably qualified persons for the post of

#### TIMBER RESEARCH OFFICER

Applicants must have a degree or equivalent pro-fessional qualification in timber or structural engineering and experience in the use of timber in the mining and other industries.

The timber research officer's duties will include making surveys of timber and forest products utilization by the Zambian industries including the mining industry, planning and executing applied research in the use of timber and forest products and liaising with industries on timber and forest products utilization.

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There is a superannuation scheme for Zambians. Non-Zambians will be paid a graduated gratuity of 20% of the total carnings in the first year. 25% in the second year and 30% in the third year of resident service. Passages will be paid for the officer, wife and minor dependent children.

Applications giving full personal details, qualifications, experience and naming three referees should be submitted to:

The Secretary General,
National Council for Scientific Research,
P.O. Box CH 158,
Chelston, Lusaka,
Zambia. (1794)

#### NEWNHAM COLLEGE TRINITY COLLEGE, CAMBRIDGE

Women chemists are invited to apply for the post of LECTURER IN INORGANIC CHEMISTRY, tenable as from October 1, 1975. Salary on pensionable scale for Cambridge University Assistant Lecturers and Lecturers: £2,393 per annum by varying annual increments to £5,402 per annum. The appointment is a joint one and will carry a Fellowship at Newnham College and a Lectureship at Trinity College. Research opportunities in the University Department of Chemistry. Further particulars may be obtained from the Principal, Newnham College. The closing date for the receipt of applications is December 31, 1974. (1854)

#### NEWNHAM COLLEGE, CAMBRIDGE

Women physicists are invited to apply for the post of LECTURER IN PHYSICS, tenable as from October 1, 1975. Salary on pensionable scale for Cambridge University Assistant Lecturers and Lecturers: £2,393 per annum by varying annual increments to £5,402 per annum. Research opportunities in the Cambridge University Department of Physics. Further particulars may be obtained from the Principal. The closing date for the receipt of applications is December 31, 1974. (1855)

#### BIOCHIMICA ET BIOPHYSICA ACTA

Applications are invited for the post of

### **EDITORIAL SECRETARY**

The BBA Editorial Secretariat, located in Amsterdam, is a small team of biochemists involved in the selection of manuscripts for publication in the world's largest biochemical journal. The work involves the choosing of reviewers for submitted articles, and subsequent correspondence with authors and editors, leading to eventual acceptance or rejection of the manuscripts. The Secretariat also maintains close contact with the journal sub-editing team, and advises on matters such as nomenclature and terminology. They thus play a central role in the quality control of the journal.

Candidates should meet the following requirements:

A wide knowledge of biochemical sciences

2-4 years of post-graduate research experience, preferably to PhD. or M.Sc. level

Initiative, decisiveness, tact and an eye for detail

An excellent command of the English language

Age under 30 and resident in Europe.

The salary for this position starts from 2,000 Dutch guilders per month depending on age and experience.

Holidays amount to 22 working days plus public holidays per year.

Applications, including curriculum vitae, should be addressed to:

The Personnel Department, Associated Scientific Publishers, P.O. Box 2400, AMSTERDAM, The Netherlands.

Further details are available on request.

(1872)

## NEW ZEALAND

Department of Scientific & Industrial Research

## SCIENTIST

#### IONOSPHERIC RESEARCH

A vacancy exists in the lonosphere Section of the Physics and Engineering Laboratory, D.S.I.R., Lower Hutt, New Zealand, for a Scientist to undertake research in electromagnetic theory with application to ionospheric radio propagation. The research programme includes investigation of radio propagation and antenna problems within New Zealand and studies of the ionosphere and magnetosphere using radio and other techniques.

Applicants should have a Ph.D. or Honours Degree in Physics or Engineering, with relevant experience.

A salary of up to \$11,153 per annum is payable, according to qualifications and experience. Further information may be obtained from the Director, Physics and Engineering Laboratory, Private Bag, Lower Hutt, New Zealand.

#### PASSAGES:

Fares for appointee and his wife and family, will be paid.

#### INCIDENTAL EXPENSES:

Up to \$120 for a single man and \$800 for a married man can be claimed to cover the cost of taking personal effects to New Zealand.

Application forms and general information are available from the High Commissioner for New Zealand, New Zealand House, Haymarket, London SW1Y 4TQ, with whom applications will close on January 5, 1975

Please quote reference PT 136 when enquiring.

(1924)

#### UNIVERSITY OF SINGAPORE CHEMICAL ENGINEERING

The University invites applications for teaching appointments in Chemical Engineering. Applicants should possess appropriate qualifications in Chemical Engineering and should have had relevant teaching/research/industrial experience. Appointments may be offered at Lecturer, Senior Lecturer, Associate Professor or full Professor level. Gross monthly emoluments in the range from S\$1,310 to S\$4,190 approx., the initial amount depending on the candidate's qualifications and experience and the level of appointment offered. The gross monthly emoluments comprise basic salary and the National Wages Council wage allowances. In addition, the University pays a 13th month annual allowance of one month's basic salary in December of each year; and contributes to the member's provident fund at 15% of basic salary and allowances. Leave, medical, housing and other benefits are also available. Candidates should write to: The Registrar, University of Singapore 10, giving curriculum vitae (bio-data), with full personal particulars, and also the names and addresses of three referees.

Rate of exchange, approx. Stg £1=S\$5.60.

(1891)

## The British Council

invites applications for the following posts

Lecturer in Lipid Biochemistry (BRAZIL)

Lecturer in Enzyme Biochemistry (BRAZIL)

Federal University of Pernambuco, Recife. Doctorate and university teaching experience required. Salary: £5,760 p.a. approx. Benefits: Medical scheme. Two-year contract. 74 BU 75, 76.

#### Lecturer in Digestive Physiology (BRAZIL)

Federal University of Pernambuco, Recife, To undertake and supervise research. Doctorate and experience in supervising research. Salary: £4,363 to £5,970 p.a. approx. Two-year contract. 74 BU 81.

Return fares are paid. Local contracts are guaranteed by the British Council.

Please write, quoting the relevant reference number, for further details and application form to: The British Council (Appointments), 65 Davies Street, London WIY 2AA. (1908)

Lister Institute of Preventive Medicine (University of London) Elstree, Herts.

## **PRODUCTION MANAGER**

The Lister Institute requires a Manager to take charge of the production for human use of bacterial vaccines, virus vaccines and therapeutic sera at its Elstree Laboratories. Applicants must have considerable experience in the organisation and day to day running of a unit producing biological materials; and the person appointed will play a major part in planning and implementing a considerable expansion in production. The salary will be in accordance with experience and qualifications and will be appropriate to a post of this seniority. Superannuation under F.S.S.U.

Applications to: The Secretary,

Lister Institute of Preventive Medicine,

Elstree, Hertfordshire.

(1914)

#### CANADIAN RED CROSS MEMORIAL HOSPITAL

Taplow, Nr Maidenhead, Berks

Research Assistant interested in the separation of proteins and their immunopathology in concection with the rheumatic diseases required at M.R.C. Rheumatism Unit at the above Hospital. Appointment at Technician or Junior Technical Officer grade.
Applications, with names of two referees, to

the Director.
CLINICAL IMMUNOLOGY
Technician required for Clinical Immunology
research in Rheumatism and connective tissue

disease.

Modern Immunological laboratory procedures are in everyday use, including immunofluorescence, immunoperoxide and radio-isotope methods.

The successful applicant will be offered training in these as. required. The post offers excellent opportunities to work for higher qualifications. I.M.L.T. or equivalent qualifications required.

Applications, with names of two referees, to the Director, M.R.C. Rheumatism Unit at the above Hospital.

(1853)

#### WEST NATIONAL SCHOOL OF **MEDICINE** (University of Wales)

DEPARTMENT OF HAEMATOLOGY SCIENTIFIC OFFICER

SCIENTIFIC OFFICER

Applications are invited from Biological Sciences Graduates for a position primarily concerned with the bioassay of erythropotetin using an established tissue culture technique. This study will form part of a clinical research programme investigating the changes that occur in the hormonal control of erythropoiesis during various haematological disturbances.

Previous experience in 'haematology is not essential but some knowledge of tissue culture techniques would be an advantage. The successful candidate may have the opportunity of studying for a higher degree.

The appointment will be at either Junior Scientific Officer or Scientific Officer Grade (£1,689 to £2,994 per annum) depending on qualifications and experience, for an initial period of two years.

years.
Application forms and further details from the Registrar, The Welsh National School of Medicine, Heath Park, Cardiff, CF4 4XN, quoting Ref. No. H24/2/8. The department may be visited by arrangement with Dr. C. D. R. Dunn, Haematology Department, Tel: Cardiff 755944 Extn. 2370.

#### **CHARING CROSS HOSPITAL** MEDICAL SCHOOL RESEARCH ASSISTANT

required for the Endocrine Laboratory of the Department of Chemical Pathology to carry out assays in connection with research into vitamin D metabolism in chronic renal failure. Experience with radioimmunoassay and/or competitive protein binding techniques helpful but not essential. Appointment for one year, renewable. Salary according to qualifications and experience. Applications and enquiries to Dr J. R. Daly, Dept of Chemical Pathology, Charing Cross Hospital Medical School, London W6 8RF.

#### University of Manchester

#### DIPLOMA IN BACTERIOLOGY

Applications are invited for admission to Applications are invited for admission to the Diploma in Bacteriology course to be held in the session 1975/76. The course is designed to give training and experience in aspects of bacteriology and animal virology which are of general importance in the application of the subject to different special fields. Application that shall find the Application of the subject to different special fields. ised fields. Applicants must have qualified in medicine, veterinary medicine, or other branches of science, and have had substantial postgraduate practical experience in bacteri-ology. Medical Research Council awards may be available to suitably qualified British subjects resident in the United Kingdom.

Forms of application may be obtained from the Department of Bacteriology and Virology, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT and should be returned as soon as possible, and in any case not later than February 1, 1975.

(1890)

#### YORK UNIVERSITY DEPARTMENT OF CHEMISTRY

DEPARTMENT OF CHEMISTRY

Applications are invited for a senior position available July 1, 1975, in the field of chemical physics with experience in laser photochemistry. The successful candidate will play a leading role in establishing a major research centre in this field. The appointment will be made at the rank of Full or Associate Professor. Salary negotiable and commensurate with rank, Send curriculum vitae, reprints and names of three referees to Professor G. O. Aspinall, Department of Chemistry, York University, Downsview, Toronto, Ontario M3J 1P3, Canada. (1863)

#### MONASH UNIVERSITY Melbourne, Australia DEPARTMENT OF GENETICS LECTURER

LECTURER

The Department already has appointments in microbial genetics, population genetics and cytogenetics, but for this appointment preference will be given to applicants who are experienced in population genetics or cytogenetics. The successful applicant will be expected to initiate and supervise research and conduct classes in the general area of his specialty as well as assisting in the general teaching programme of the Department.

Salary Scale: \$A9,002 to \$A12,352 per annum with superannuation based on an endowment assurance scheme, the employee and employer contributing 5% and 10% respectively.

Benefits: travelling expenses for appointee and family; removal allowance; repatriation after three years' appointment if desired; temporary housing for an initial period; availability of loans for house purchase; study leave entitlement accumulates at the rate of one month's leave for each six months' service up to six years, with provision for financial assistance.

Further general information and details of application procedure are available from the Academic Registrar, Monash University, Wellington Road, Clayton, Victoria 3168, Australia, or the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WCIH Enquiries about departmental research interests and facilities to Professor B. W. Holloway in the

Enquiries about departmental research interests and facilities to Professor B. W. Holloway in the

and facilities to Professor D. W. Holloway III III.
University.
Closing Date: December 7, 1974.
The University reserves the right to make no appointment or to appoint by invitation. (1868)

#### UNIVERSITY OF AUCKLAND New Zealand

Applications are invited for the following appointments:

#### LECTURESHIP IN CIVIL **ENGINEERING**

Applicants should have an Honours degree or its equivalent. Although not essential, professional experience is desired. Opportunities are available for the pursuit of a higher degree.

#### SENIOR LECTURESHIP/ LECTURESHIP IN ELECTRICAL ENGINEERING

Well-qualified applicants in any field of electrical engineering will be welcomed, but, preference will be given to applicants with teaching and research experience in electromagnetic fields and radio antennae, or in control systems; possession of a higher degree and industrial experience would be advantageous advantageous

#### LECTURESHIP IN MECHANICAL ENGINEERING

Applicants should have qualifications and experience in one of the following special areas—Control engineering; Dynamics; Industrial thermo-

Control engineering; Dynamics; inquisition dynamics.

Salary scales: Commencing salary within the appropriate scale will be determined in accordance with qualifications and experience. Senior Lecturer NZ59.503 to NZ511,153. In exceptional cases the Council may extend this scale up to NZ512,142.

Additional information, conditions of appointment and application procedure is obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WCIH OPF.

Applications, close on December 7, 1974.

(1866)

#### GEOGRAPHY GRADUATE

(female) required for administrative work and occasional teaching duties. Salary according to age and experience on the scale £1,055 & £1,646, plus full residential benefits. Please write to F. J. Bingley (Warden and Director of Studies), Flatford Mill Field Centre, East Bergholt, nr. Colchester, CO7 6UL. (1873)

### **Senior Pharmacologist Anti-Inflammatory Research**

Applications are invited for a senior post in our Pharmacology Department in Welwyn Garden City, Hertfordshire. The department is responsible for carrying our research and screening work aimed at the development of new drugs in which the pharmacologists participate in the research programmes of interdisciplinary groups.

The senior pharmacologist we appoint will be responsible for directing the work of a team of pharmacologists engaged in anti-inflammatory research. Applicants should have a Ph.D. qualification and preferably some post-doctoral experience in anti-inflammatory research.

Our Pharmacology Department is located in new laboratories where the facilities and working conditions are excellent. Conditions of service are above average and in appropriate cases generous assistance with relocation will be available.

Roche Products Limited is part of a major international pharmaceutical firm based in Switzerland and is itself one of the leaders in the industry in the United Kingdom. If you would like to apply for this post please write for further information, a Company booklet, to the Welwyn Personnel Manager.

Closing date for applications, December 13, 1974.



Roche Products Limited Welwyn Garden City Hertfordshire AL7 3AY (1896)

EVANS BIOLOGICALS LIMITED

## Virologist

Evans Biologicals is a leading company in the manufacture of viral and bacterial vaccines for human and veterinary use and is a member of the Glaxo group of companies. Its modern premises are situated in Speke on the outskirts of Liverpool within commuter distance of Cheshire and the Wirral and the Lancashire coast.

We require a graduate wirologist or a senior technician with some postgraduate experience either in academic or industrial research to join our Research and Development team. The initial project will be the development of recombinant strains of influenza viruses and investigation of their replication potential.

An attractive salary according to age and experience will be offered and in addition there is a bonus scheme related to the profits of the Glaxo Group. Assistance with relocation expenses will be given in appropriate

Please reply, with brief details of your career to:



Dr. M. C. Cook, Management Staff Adviser, Evans Medical Limited, Speke. Liverpool L24 9JD.

## May & Baker require a

The applicant should have several years' post-graduate biochemical experience and although a Ph.D. is not essential, the candidate will be required to give evidence of research ability.

He or she will pursue basic biochemical research and will also be concerned with the development of new tests for the detection of potentially carcinogenic compounds.

Publication of appropriate work is encouraged, likewise attendance at scientific meetings. There are active links with a number of university departments and hospital personnel.

Various attractive residential areas are easily accessible from the Research Institute and help with removal expenses will be provided

Write to the Personnel Manager, May & Baker Ltd., Dagenham, Essex. RM 107XS (quoting reference number 265/N/I), or telephone Elizabeth Jones on 01-592 3060 ext. 506 for further information and an application form. .



(1918)

## **High Energy Physics** Programmer

The High Energy Physics Division of the Rutherford Laboratory has a vacancy in its Bubble Chamber Research Group for a Physicist Programmer. Applications are invited from candidates with preferably a background in particle physics together with experience in hardware and/or small computer software. Duties will include responsibility for the design of the interfacing and software for a spark chamber trigger system associated with a small computer on line to the rapid cycling bubble chamber presently being built at the Rutherford Laboratory. The overall responsibility for a PDP 8E system on line to film measuring machines will also be part of the duties. The appointment will be made in the grade of Senior Scientific Officer.

#### QUALIFICATIONS

A 1st or 2nd class honours degree with 4 years post-graduate experience.

£3,380 rising to a maximum of £4,755 (plus threshold payments).

Please write or telephone for an application form to:-Mr D. Williams, Rutherford Laboratory, Chilton, Didcot, Oxon OX11 0QX

Telephone: Abingdon 1900, extension 510

Quote Reference: VN608R/34 Closing Date: 2 Dec. 1974

#### RUTHERFORD LABORATORY

Science Research Council

#### THE QUEEN'S UNIVERSITY OF BELFAST

#### Temporary Lectureship

DEPARTMENT OF CHEMISTRY

Applications are invited for a temporary lectureship in Organic Chemistry tenable from January 1, Chemistry tenable from January 1, 1975 to September 30, 1977 with a possible extension to December 31, 1977. The salary scale is £2,118 to £4,896 with contributory pension rights under the F.S.S.U. Threshold supplements are additional to this scale. Initial placing on the scale will depend on qualifications and experience. Applications should be perience. Applications should received by December 1, 1974.

Further particulars may be obtained from The Personnel Officer, The Queen's University of Belfast, Belfast BT7 1NN, Northern Ireland (Please quote Ref. 74/N). (1902)

#### KING'S HEALTH DISTRICT (TEACHING)

LAMBETH/SOUTHWARK/LEWISHAM AREA HEALTH AUTHORITY (Teaching)

King's College Hospital Diabetic Department has a vacancy for a laboratory technician or science graduate with biochemical experience to assist in research into metabolic problems of diabetes. The work, which will include clinical as well as biochemical aspects, will be undertaken in conjunction with medical staff in the Diabetic Department.

Application forms obtainable from the Personnel Office, King's College Hospital, Denmark Hill, London S.E.5. Tel: 01-274 6222, Ext. 2726 (Miss Millwood) should be completed and returned by November 29, 1974. (1875)

#### UNIVERSITY OF SIERRA LEONE FOURAH BAY COLLEGE

Applications are invited for two positions of LECTURER IN ZOOLOGY

LECTURER IN ZOOLOGY

Preference will be given to candidates who have specialised in (i) Entomology; (ii) Physiology, and who can teach courses in these subjects up to special honours level. Salary scale (under review): Le2,400 to Le4,740 p.a. (Le2=f1 sterling). The British Expatriates Supplementation Scheme is unlikely to be applied to this appointment. F.S.S.U. Family passages; various allowances; regular overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, not later than December 13, 1974 to the Secretary, University of Sierra Leone, Private Mail Bag, Freetown, Sietra Leone. Applicants resident in U.K. should also send I copy to Inter-University council, 90/91 Tottenham Court Road, London WIP ODT. Further particulars may be obtained from either address. (1877)

#### UNIVERSITY OF SIERRA LEONE FOURAH BAY COLLEGE

FOURAH BAY COLLEGE

Applications are invited for the post of ASSO-CIATE PROFESSOR IN ORGANIC CHEMISTRY, tenable from September 1, 1975. Appointee would be expected to give courses to general and honours degree levels in three or more of the following topics: the chemistry of Natural Products; Structural Organic Chemistry; Stereo-chemistry; Organic Reaction Mechanisms; Classical Organic Chemistry, Salary scale (under review): Le5,500 to Le5,600 p.a. (f1 sterling=Le2). The British Government may supplement salary by £2,550 p.a. (sterling) for married appointees or £1,554 p.a. (sterling) for single appointees (normally free of all tax) and provide children's education allowances and holiday visit passages; F.S.S.U. Family passages; various allowances; regular overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, not later than December 13, 1974 to the Secretary, University of Sierra Leone, Private Mail Bag, Freetown, Sierra Leone, Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London WIP 0DT. Further particulars may be obtained from either address. (1879)

#### UNIVERSITY OF MALAWI BUNDA COLLEGE OF AGRICULTURE

Applications are invited for LECTURESHIP IN SOIL SCIENCE IN THE CROP PRODUCTION DEPARTMENT. Applicants should have a good honours degree with postgraduate qualifications and experience in Soil Science. Some general agronomic or ecological background and interest in tropical soils would be an advantage. Duties include teaching Soil Science as applied to agriculture at both degree and diploma level and the development of practical research programme relevant to the agricultural needs of Malawi.

practical research programme relevant to the agricultural needs of Malawi.

Salary scale (including expatriate addition): K2,809 to K4,714 p.a. plus either a University Addition of K600 p.a. (taxable in Malawi) or supplementation (normally tax free) in appropriate cases, by the British Government, in range £900 to £1,320 p.a. (sterling) (if married) or £444 to £864 p.a. (sterling) (if single) (normally free of all tax). (£1 sterling=K1.20). There may also be provision of children's education allowances and holiday visit passages. Gratuity; superannuation scheme transferable with F.S.S.U.; family passages; various allowances; biennial overseas leave; housing. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by air mail, not later than December 9, 1974, to the Registrar, University of Malawi, University Office, P.O. Box 278, Zomba, Malawi, Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P ODT. Further particulars may be obtained from either address." (1878)

#### UNIVERSITY OF DAR ES SALAAM TANZANIA

Applications are invited for the following posts in the DEPARTMENT OF SOIL SCIENCE AND AGRICULTURAL CHEMISTRY:

#### PROFESSOR OF SOIL SCIENCE

Applicants should have a Ph.D., or equivalent research experience, in any ONE of the following: Soil Physics; Soil Chemistry; Soil Fertility; Plant Nutrition; Pedology. Previous university teaching experience is desirable but not essential.

#### SENIOR LECTURER/LECTURER IN BIOCHEMISTRY (Agriculture)

Applicants should have a Ph.D. in Biochemistry or equivalent research experience. Preference will be given to those whose first degree is in Chemistry rather than in Agriculture, Appoince will be required to teach Biochemistry and some related aspects of Physical Chemistry to undergraduates, studying for the B.Sc.(Agric.) and B.Sc.(Forestry). He will be expected to conduct research and direct postgraduate studies in Biochemistry.

Salary scales Professor T£3,332 to T£3,662 p.a. Senior Lecturer T£2,632 to T£3,052 p.a. Lecturer T£2,150 to T£2,570 p.a. (T£1=£1,19 sterling). The British Government may supplement salaries in range £996 to £2,448 p.a. (sterling) for married appointees or £348 to £1,500 p.a. (sterling) for single appointees (normally free of all tax) and provide children's education allowances and holiday visit passages. F.S.S.U. Family passages; biennial overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by air mail, not Jater than December 11, 1974 to the Chief Academic Officer, University of Dar es Salaam, P.O. Box 35091, Dar es Salaam, Tanzania. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London WIP ODT. Further particulars may be obtained from either address.

#### UNIVERSITY OF DAR ES SALAAM TANZANIA

Applications are invited for the post of

#### PROFESSOR IN MATHEMATICS

PROFESSOR IN MATHEMATICS

Applicants must hold a doctorate. Appointee should have experience over a wide range of Mathematics and its applications, and he should be strongly interested in the development and encouragement of Mathematical curricula at all levels relevant to a rapidly developing country. Salary scale: T£3,332 to T£3,662 p.a. (T£1=£1.19 sterling). The British Government may supplement salary by £2,448 p.a. (sterling) for married appointee or £1,500 p.a. (sterling) for single appointee (normally free of all tax) and provide children's education allowances and holiday visit passages. F.S.S.U. Family passages; biennial overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by air mail, not later than December 11, 1974 to the Chief Academic Officer, University of Dar es Salaam, P.O. Box 35091, Dar es Salaam, Tanzania. Applicants resident in U.K. should also send I copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. (1881)

## CSIRO

#### **AUSTRALIA**

## DIVISION OF COMPUTING RESEARCH

CANBERRA, A.C.T.

## RESEARCH **SCIENTIST**

The Commonwealth Scientific and Industrial Research Organisation has a broad charter for research into primary and secondary industry areas. The Organisation has approximately 6,500 employees—2,100 of whom are research and professional scientists-located in Divisions and Sections throughout Australia.

FIELD:

## **PICTURE PROCESSING**

GENERAL: The Division of Computing Research, with headquarters in Canberra, A.C.T., operates a computer system based on a CDC Cyber 76 computer. An expanding network of mini-computers at CSIRO research centres enables the concentration of interactive and batch work in Canberra. Currently about thirty batch terminals and more than one hundred and fifty interactive terminals are connected.

The capacity and potential of the computer system provide an excellent tool for research and development projects. Research studies at present include picture processing, simulation techniques, operating systems, computer languages and data-base management.

Additional facilities for processing pictures on the computer system are being acquired. In particular, an image-dissector scanner and a terminal for displaying grey-scale images will be interfaced to the system in Canberra. This equipment will be used to develop picture processing software for a variety of applications and will assist current research on the evaluation of techniques for picture interpretation and machine perception.

DUTIES: To conduct research on techniques for picture processing and to participate in the development of associated facilities.

QUALIFICATIONS: A Ph.D. degree in an appropriate field or equivalent qualifications together with demonstrable research ability.

SALARY: The appointment will be made within the salary ranges of Research Scientist or Senior Research Scientist: \$A10,778 to \$A16,187 p.a.

TENURE: The position is available for an indefinite period and Australian Government Superannuation benefits are available.

Applications stating full personal and professional details, the names of at least two professional referees and quoting Reference Number 900/261, should reach:-

The Personnel Officer, Australian Scientific Liaison Office, 64-78 Kingsway, LONDON WC2B 6BD

by the 13th December, 1974.

Applications in U.S.A. and Canada should be sent to The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W., WASHINGTON, D.C. 20036, U.S.A.

(1930)

#### CSIRO

#### **AUSTRALIA**

#### DIVISION OF APPLIED **GEOMECHANICS**

SYNDAL, VIC.

## RESEARCH SCIENTIST

The Commonwealth Scientific and Industrial Research Organisation has a broad charter for research into primary and secondary industry areas. The Organisation has approximately 6,500 employees-2,100 of whom are research and professional scientists-located in Divisions and Sections throughout Australia.

#### FIELD:

## COASTAL AND OCEAN ENGINEERING

GENERAL: The Division of Applied Geomechanics undertakes a wide range of studies in rock mechanics and soil mechanics. An expansion of the Division's programme of coastal investigations is currently under way to meet the substantial expansion of industrial, offshore mining and other engineering activities which can be foreshadowed along the Australian coastline and offshore on the Australian continental shelf.

DUTIES: To join a research team to study and categorise the physical problems of the Australian coast and continental shelf, and to formulate basic multidisciplinary research projects to facilitate future engineering developments in the application of geomechanics.

QUALIFICATIONS: A Ph.D. degree or equivalent qualification in the engineering field, together with demonstrable research ability and an adequate understanding of the physical branches of marine science and an appreciation of the complex ecology of coastal and continental shelf areas.

SALARY: Appointment will be made within the salary ranges of Research Scientist or Senior Research Scientist: \$A10,778 to \$A16,187 p.a. Consideration will be given to appointment at a higher level for an outstanding applicant.

TENURE: Either an indefinite or a fixed term appointment may be negotiated. Indefinite appointments carry Australian Government Superannuation benefits.

Applications stating full personal and professional details, the names of at least two professional referees, and quoting Reference Number 920/188, should reach:-

The Personnel Officer. Australian Scientific Liaison Office, 64-78 Kingsway, LONDON WC2B 6BD

by the 13th December, 1974.

Applications in U.S.A. and Canada should be sent to The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W., WASHINGTON, D.C. 20036, U.S.A.

(1931)

#### **TECHNICIAN**

for work on the effects of carcinogens on protein and nucleic acid metabolism. Salary on Whitley Council scale (£1,986 to £2,445 including London Weighting) depending on experience. Applications in writing giving details of experience, qualifications and names of two referees to The Director (N), Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London WIP 5PR. (1882)

#### UNIVERSITY COLLEGE LONDON DEPARTMENT OF BIOPHYSICS ELECTRON-MICROSCOPIST

RESEARCH ASSISTANT required to work with Professor R. Miledi on problems in Neurobiology, which forms part of a long-term Project supported by the M.R.C.

Preference given to applicants with experience of freeze-etching or analytical electron-microscopy. Salary within Lecturer scale plus London Allow-

Applications with curriculum vitae and names and addresses of two referees to Mrs K. Garnons-Williams, Biophysics Dept. (N), University College London, Gower Street, London WCIE 6BT. (1883)

#### THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

(University of London)

COURTAULD INSTITUTE OF BIOCHEMISTRY Applications are invited for a Research Assistant post in the Steroid Unit of the Institute. Candidates should be at Doctorate level with a sound basis in Chemistry and Biochemistry. Salary according to age and experience within the range £2,931 to £3,285 plus London allowance, currently £213 per annum and superannuation under F.S.S.U. Applications giving details of academic achievement and the names and addresses of two referees to the Director, Courtauld Institute of Biochemistry, The Middlessex Hospital Medical School, London W1P 5PR, by December 2, 1974 quoting (C.R.C. Biochem 31).

#### THE HORMEL INSTITUTE OF THE UNIVERSITY OF MINNESOTA

invites applications from qualified individuals for the position of EXECUTIVE DIRECTOR of The Hormel Institute which is located in Austin, Minnesota. The Executive Director is responsible for overall administration of an institute with 80-90 employees, including 20 of whom have academic rank. Candidates for this position should be recognised authorities in the lipid field, and should have at least 12 years of research experience in the field. The director is expected to maintain an active research programme.

Closing date for receipt of all materials is February 1, 1975. Send curriculum vitae, a list of publications and names of three referees to Dr J. E. Gander (Chairman, Search Committee), Department of Biochemistry, University of Minnesota, St Paul, MN 55108. (1886)

#### **QUEENS COLLEGE** OF THE CITY UNIVERSITY OF NEW YORK ASSISTANT PROFESSOR

position available for 1975/76 academic year, with possibility of reappointment. Ph.D. required. Send curriculum vitae to Chairman, Dept of Earth and Environmental Sciences, Queens College, Flushing, New York 11367. An Affirmative Action/Equal Opportunity Employer. (1888)

#### THE DEPARTMENT OF BIOLOGICAL SCIENCES

of the

#### UNIVERSITY OF SOUTHERN **CALIFORNIA**

CALIFORNIA

has three vacancies in Ecology and Evolution. One is in the area of marine ecosystems, especially modelling and mathematical ecology. One is for a marine population ecologist with major interest in phytoplankton. The third full-aime faculty position offered in conjunction with the Allan Hancock Foundation is for an Evolutionary biologist with interest in a major invertebrate marine group. The position involves some responsibility for the Foundation's research collection, and applications are encouraged with interest in biochemical evolution. Send curriculum vitae to Dr Bernard C. Abbott, University of Southern California, Los Angeles, CA 9007, U.S.A. An Equal Opportunity/Affirmative Action Employer. (1889)

#### ROYAL POSTGRADUATE MEDICAL SCHOOL

TECHNICAL OFFICER (2 posts) required for Department of Clinical Pharmacology to join groups working on

- (1) The role of catechol amines in essential hypertension and
- The pharmacology and metabolism of new drugs in man.

Salary on scale £2,268 to £3,729 per annum plus threshold payment, according to age and experience.

Applications to the Personnel Officer (743 2030 Ext. 93), R.P.M.S., Hammersmith Hospital, DuCane Road, London W12 0HS, quoting ref. o. 20/(1) or (2).

#### UNIVERSITY OF NEWCASTLE UPON TYNE

NORTHERN COKE RESEARCH LABORATORY

RESEARCH LABORATORY

Applications are invited for a post of Senior Research Associate (from £2,580) or Research Associate (from £2,118) in the Laboratory for research on fundamental problems connected with the strength of coke and its dependence on its precursors and mode of formation. The Laboratory is within the School of Chemistry and the post is suitable for any candidate interested in Material Science, irrespective of initial degree. It is available until September 30, 1976 in the first instance, commencing January 1, 1975, or by arrangement. Starting salary according to age and experience.

Applications including a brief curriculum with

Applications including a brief curriculum vitae and the names of two referees should be sent as soon as possible to the Honorary Director, Professor D. H. Whiffen, School of Chemistry University of Newcastle upon Tyne, Newcastle upon Tyne, NEI 7RU. (1895)

#### ORGANIC CHEMIST (post Ph.D.)

required for work in synthesising steroid and other derivatives for use as immunogens and for labelling with radioiodine for use in Radioimmunoassay systems. Interest in microanalytical work and in medical application desirable.

Appointment for three years on salary scale £2,019 to £3,636 per annum.

Applications, including the names of tw referees, to Dr. W. M. Hunter, Head, M.R.C. Radioimmunoassay Team, 2 Forrest Road, Ediburgh EH1 2QW. (1899)

#### NUFFIELD INSTITUTE FOR MEDICAL RESEARCH

A vacancy has arisen for a postgraduate student to work on electromagnetic flowmeter development for a period of up to 3 years. Candidates must be British subjects and have either a first class or upper second class degree in Physics or Engineering. Apply to Dr. D. G. Wyatt, Headley Way, Headington, Oxford. (1900)

#### UNIVERSITY OF BRISTOL M.R.C. BRAIN AND PERCEPTION LABORATORY

#### POSTDOCTORAL RESEARCH **ASSISTANTSHIP**

for experimental work in human perception.

We are looking for candidates with initiative and originality. The area of research extends from cognitive processes to neurological disturbances, including development of novel experimental techniques.

Commencing salary up to £3,108 per annum (plus any Threshold Agreement payments applicable) according to age and experience.

Applicants should write in confidence to Professor R. L. Gregory, Brain and Perception Laboratory, Department of Anatomy, The Medical School, University Walk, Bristol BS8

Applications are invited from biology graduates for a three year post as

#### RESEARCH ASSISTANT

initially in the Pathology Department of St. George's Hospital Medical School, and thereafter at the Middlesex Hospital Medical School. The successful candidate will participate in projects using immuno-fluorescent and chemical techniques and scanning electron microscopy. Previous laboratory experience desirable. Starting salary in the region of £1,600 p.a. depending on qualifications.

Apply to Dr N. Woolf, Pathology Department, St. George's Hospital Medical School, Hyde Park Corner, London, SW1X 7NA. (1909)

#### CSIRO

#### **AUSTRALIA**

DIVISION OF WILDLIFE RESEARCH CANBERRA, A.C.T.

## **SENIOR BIOLOGIST**

The Commonwealth Scientific and Industrial Research Organisation has a broad charter for research into primary and secondary industry areas. The Organisation has approximately 6,500 employees-2,100 of whom are research and professional scientists-located in Divisions and Sections throughout Australia.

FIELD:

## **MARSUPIAL** PHYSIOLOGY

GENERAL: The Division of Wildlife Research includes some forty scientists engaged in research on biology of native and introduced birds and mammals. Its principal interests at present are in ecology and behaviour but also include parasitology and physiology.

The Division is now increasing the component of physiology in its programme and is seeking an animal physiologist to develop this work.

DUTIES: To lead the group of physiologists that is being established and to integrate their work with the ecological and other investigations.

QUALIFICATIONS: Applicants should have an outstanding record in research in marsupial physiology, preferably in either reproduction or metabolism as it is anticipated that these fields will be of major importance in the Division's total programme.

SALARY: Appointment will be made within the salary ranges of Senior Principal Research Scientist or Chief Research Scientists: \$A20,359 to \$A22,482 p.a.

TENURE: The position is available for an indefinite period and Australian Government Superannuation benefits are available. Alternatively a fixed term appointment may be negotiated.

Applications stating full personal and professional details, the names of at least two professional referees, and quoting Reference Number 560/342, should reach:-

The Personnel Officer, Australian Scientific Liaison Office, 64-78 Kingsway, LONDON WC2B 6BD

by the 13th December, 1974.

Applications in U.S.A. and Canada should be sent to The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W., WASHINGTON, D.C. 20036, U.S.A.

(1932)

### CSIRO

#### **AUSTRALIA**

#### DIVISION OF MECHANICAL **ENGINEERING**

HIGHETT, VIC.

## RESEARCH SCIENTIST

The Commonwealth Scientific and Industrial Research Organisation has a broad charter for research into primary and secondary industry areas. The Organisation has approximately 6,500 employees-2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

FIELD:

## **SOLAR ENERGY** UTILIZATION

GENERAL: The Division of Mechanical Engineering is seeking a Research Scientist to take part in the activities of the solar energy utilisation group, For a number of years the Division has conducted research in this area and current investigations include the use of solar energy as a source of heating for domestic and commercial hot water, space heating and cooling, and industrial process heating. The work involves theoretical studies, prototype development and experiments under laboratory and field conditions.

The Division also conducts extensive research in the fields of heat and mass transfer, air conditioning, environmental control and fluid dynamics.

DUTIES: To conduct research into methods of utilising solar energy as a source of heat at temperatures up to 120°C and to lead small teams oriented to specific goals within this area.

QUALIFICATIONS: A Ph.D. degree in mechanical engineering or equivalent qualifications and demonstrable research ability, preferably with appropriate postdoctoral experience. An understanding of computer simulation techniques is desirable and some background in solar energy utilisation would be an advantage.

**SALARY:** The appointment will be made within the salary ranges of Research Scientist or Senior Research Scientist: \$A10,778 to \$A16,187 p.a.

TENURE: This position is available for an indefinite period and Australian Government Superannuation benefits are available.

Applications stating full personal and professional details, the names of at least two professional referees and quoting Reference Number 430/337, should reach:-

The Personnel Officer. Australian Scientific Liaison Office. 64-68 Kingsway. LONDON WC2B 6BD

by the 13th December, 1974.

Applications in U.S.A. and Canada should be sent to The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W., WASHINGTON, D.C. 20036, U.S.A.

(1933)

#### UNIVERSITY OF WESTERN AUSTRALIA Perth

Applications are invited for appointment to a CHAIR IN CIVIL ENGINEERING.

CHAIR IN CIVIL ENGINEERING.

This is one of two Chairs in the Department of Civil Engineering, the other being held by the current head, Professor D. H. Clyde. The Department is one of three in the Faculty of Engineering, the other two being Electrical and Electronic Engineering and Mechanical Engineering, Preference will be given to applicants with special interests in the water or the geotechnical fields, but applications will be considered from all candidates who, if appointed, would make major contributions to the University and the community through their specialities. Detailed information on the teaching and research activities of the Department and conditions of appointment may be obtained from the Staffing Officer.

The headship of University departments, in particular the tenure of headships, is at present under review and it is likely that these will become fixed term appointments from within departments.

The current salary for a Professor is SA19,614 p.a. Benefits include superannuation similar to F.S.S.U., fares to Perth for appointee and dependent family, removal allowance, study leave and long service leave and housing loan scheme.

Applications in duplicate stating full personal particulars, qualifications, experience and the names and addresses of three referees should reach the Staffing Officer, University of Western Australia, Nedlands, Western Australia, 6009, by December 14, 1974.

Further particulars and conditions of appointment available from Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WCIH OPF. This is one of two Chairs in the Department

#### VICTORIA UNIVERSITY OF WELLINGTON

New Zealand

#### LECTURESHIP IN CHEMISTRY

Applications are invited for the above-mentioned Applications are invited for the above-mentioned post. Applicants should preferably have experience in, or be prepared to develop expertise in, areas relevant to courses of analytical chemistry, isotope chemistry, industrial chemistry, especially related to New Zealand industry (including the organization and supervision of vacation field trips), a new service course in materials science for architecture students, and other applied areas.

Salary range: NZ\$7,361 to \$9,339 p.a.

Further particulars and application procedure from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF.

Applications close on November 30, 1974.

Applications close on November 30, 1974. (1906)

#### DEPARTMENT OF PHYSICS UNIVERSITY OF ALBERTA ELECTRONMICROSCOPIST

Applications are invited for an Assistant Professor in the area of Electronmicroscopy. Exceptional candidates in other areas of condensed state physics will be considered. The condensed state group at the University of Alberta consists of eight members in a Physics Department of forty-two faculty members.

The effective date of appointment is April 1, 1975. The closing date for applications is February 1, 1975.
Send vitae. list of publications

1, 1975.
Send vitae, list of publications, and names of three referees to:
Dr J. T. Sample
Chairman
Department of Physics
University of Alberta
Edmonton, Alberta, T6G 2E1

(X1911)

#### LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

(University of London)
KEPPEL STREET, WC1E 7HT

WINCHES FARM FIELD STATION, ST. ALBANS

A Senior Laboratory Technician is required to work at St. Albans on a project on the immunology of schistosomiasis, in collaboration with parasitologists investigating vaccination against this disease.

this disease.

Candidates should preferably be graduates or hold F.I.M.L.T./H.N.D. and have experience of immunological work. Salary, depending on qualification and experience, in the range £2,238 to £2,835 plus threshold payments.

Applications in writing, giving details of career etc., to Secretary (A1) N at the London address by December 21, 1974. Further information may be obtained from Dr. M. G. Taylor (St. Albans 53858).

#### UNIVERSITY OF HONG KONG LECTURESHIP/ASSISTANT LECTURESHIP IN BIOCHEMISTRY

Applications are invited for a post of Lecturer/ Assistant Lecturer in Biochemistry. An interest in Molecular Genetics or experience in immuno-logical methods would be an advantage but neither is regarded as essential.

Annual salaries (currently under review and superannuable) are:— Lecturer: HK\$44,040 by 2,940 to 49,920 BAR 52,860 by 2,940 to 61,680 by 3,000 to 73,680; Assistant Lecturer: HK\$32,280 by 2,940 to 41,100. At the time of issue the rate of exchange is £1=HK\$12.00 approx.

The entry point to the salary scale will depend on qualifications and experience: for example, a medically-qualified appointee who has completed his pre-registration year will be appointed as a Lecturer at the minimum point. Salaries for female appointees below HK\$67,680 are slightly lower than given above; equalization of male and female salary scales will be achieved by April 1, 1975.

Candidates who applied earlier in the year will be reconsidered and need not apply again but may submit further material in support of their applications if they wish.

Further particulars and application forms may be obtained from the Association of Commonwealth Universities (Appts.), 36. Gordon Square, London WCIH OPF, or the Secretary to the Council, University of Hong Kong.

Closing date for applications is December 20, 1974. (1907)

#### UNIVERSITY OF EAST ANGLIA LECTURERS IN BIOLOGY **ECOLOGY AND** DEVELOPMENTAL BIOLOGY

Applications are invited for appointments as LECTURER in the School of Biological Sciences to begin not later than September 1, 1975, in the fields of ecology and developmental biology. Salary within the range £2,118 to £2,757 on the scale £2,118 to £4,896, plus threshold payments and F.S.S.U.

Details from the Establishment Officer, University of East Anglia, Norwich NR4 7TJ, with whom applications (one copy only) together with the names and addresses of three persons to whom reference may be made, should be lodged not later than December 9, 1974. (1913)

#### **GRIFFITH UNIVERSITY** BRISBANE, AUSTRALIA SCHOOL OF SCIENCE

In the early years the main areas of concentration of the School will be experimental physics, chemistry, and biochemistry. Applicants for posts 1 and 2 should be prepared to be associated with interdisciplinary research projects in one or more of these areas, as well as pursuing their own research interests. Applications are invited from men and women for the positions described below to be available for appointment, in the case of posts 1 and 2, on or about September 1, 1975, in the case of post 3 on or about January 1, 1975.

1. LECTURESHIP—BIOLOGICAL ORGANIC CHEMISTRY: The appointee's initial teaching responsibilities will be in the planning of the second year chemistry courses, both theoretical and practical. Candidates should have research experience in some biological aspect of organic chemistry

experience in some biological aspect of organic chemistry.

2. LECTURESHIP—APPLIED | MATHEMATICS: (Theoretical Biologist/Chemist/Physicist): Applicants must have strong mathematical expertise and an interest in scientific problems. Preference may be given to applicants with a research interest in biological problems, but this is not a requirement. The initial teaching responsibility of the appointee would be in teaching mathematics for second year science students.

3. RESEARCH FELLOWSHIP — ORGANIC CHEMISTRY: A post-doctoral research fellowship is available, for one year in the first instance, for work on "Co-polymers of Sucrose Derivatives", funded by the International Sugar Research Foundation, and under the general direction of Professor R. D. Guthrie. Applicants should have experience in synthetic organic chemistry, or in polymer chemistry.

Salaries: Lecturer SA9,002 to \$12,352; Research Fellow \$A7,454 to \$7,837.

Further details and the method of application for nosts 1 and 2 for which the closing date is

Further details and the method of application for posts 1 and 2, for which the closing date is **December 2**, 1974, can be obtained from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WCIH 0PF.

Applications for post 3 should be made, as soon as possible, directly to Professor R. D. Guthrie, School of Science, Criffith University, Nathan, Brisbane, Quensland, Australia 4111, and should contain the names of two referees.

#### QUEEN ELIZABETH COLLEGE (UNIVERSITY OF LONDON) CAMPDEN HILL ROAD, W8 7AH TEMPORARY RESEARCH POST

Chemist/Biochemist required in Microbiology Department, January to September 1975 to assist in a isolation of serum protein growth factor required by human cells in culture. Salary at rate of £1,761 p.a. plus threshold payments.

Apply to Professor S. J. Pirt. (1922)

#### THE POLYTECHNIC WOLVERHAMPTON

#### DEPARTMENT OF BIOLOGICAL SCIENCES LABORATORY TECHNICIANS

(3 posts)

Preference will be given to candidates with interests in one of the following subject areas:—Biochemistry, Plant. or Animal Physiology and Plant Pathology or Microbiology. Duties would involve the servicing of laboratories for undergraduate practical classes.

Salary on Scale Grade T1 £807 to £1,866 commencing point dependent upon age and experience. Additions to scale for current threshold allowances and recognised qualification.

Application form and details from:—

The Establishment Officer

The Polytechnic

Wolverhampton WV1 1LY.

Wolverhampton WV1 1LY.

(1923)

#### GRADUATE BIOCHEMIST

required by Dept. of Experimental Pathology twork on immunochemical and electron microscopis studies in glomerulonephritis. Apply, with detail of experience and names and addresses of two referees to, The Secretary, St. Mary's Hospita Medical School, Paddington, London W2 IPG Ref. EPBG. (1943)

#### THE ROYAL DENTAL HOSPITAL OF LONDON

SCHOOL OF DENTAL SURGERY (University of London) 32 Leicester Square, W.C.2.

32 Leicester Square, W.C.2.

Applications are invited for the appointment of Graduate Research Assistant in the Department of Physical Sciences on either a full-time or part-time basis. Applicants must hold a good degree in Physics, Chemistry or a related subject but previous research experience is not essential. In the case of a full time appointment, the possibility of extension and would permit registration for a higher degree as a part-time internal student of the University of London. Salary range, according to age and experience, from £1,606 to £1,902 plus London Allowance at £213 p.a. and Threshold Agreement Payments and F.S.S.U. benefits.

Further particulars may be obtained from Dr. N. E. Waters and applications should be sent to the School Secretary. (1944)



Applications are invited from suitably qualified personnel for the following vacancy in the Technical Services Department of Mount Isa Mines Limited.

### RESEARCH CHEMIST

#### **Location: Mount Isa** Queensland, AUSTRALIA

Ref. 9274

The successful applicant will be appointed to the X-ray analysis group in the chemical laboratory. The laboratory is equipped with a Philips PW1212 sequential spectrometer, two Philips PW1270 simultaneous spectrometers and a PDP-11 computer. The appointee will be required to maintain the quality of routine analyses on a continuous 24 hour basis and to expand and improve existing product/element calibrations and will also be required to assist in and interest and the product which the product is sufficient to the product that the product which the product is sufficient to the product that the product the product the product that in, and initiate, development work which may include investigations into sample and specimen preparation techniques, new calibration methods, mathematical projects and computer programming.

QUALIFICATIONS AND EXPERIENCE: Applicants must possess a degree or diploma in science and have a minimum of four years' practical industrial experience. Prior experience in X-ray analysis is essential.

REMUNERATION: Will depend on qualifications and experience. However, on commencement total remuneration including bonus, prosperity and annual leave loadings, production payment and holiday air fares is envisaged to be in the vicinity of \$A10,000 to \$A11,500 per annum. Salaries are reviewed annually and Zone "A" taxation

#### **GENERAL CONDITIONS:**

- · Married and single accommodation available.
- Assistance will be given with fares and removal expenses.
- · Six weeks and two days' annual leave and liberal sick leave with
- · Refund of medical expenses.
- · Immediate participation in superannuation plan.
- · Credit union facilities are available for new employees.

Applications should be addressed to Mr. T. B. A. Roberts

#### M.I.M. HOLDINGS LIMITED

Adelaide House, King William Street London EC4 9DX

or by phoning London 01 62 67446 for an application form.

4654

#### UNIVERSITY OF BRISTOL DEPARTMENT OF PATHOLOGY

#### REPRODUCTIVE IMMUNOLOGY GROUP

#### POST-DOCTORAL RESEARCH ASSISTANT/ASSOCIATE

Applications are invited for a further post within an established research group investigating immunological aspects of reproduction and development in laboratory rodents. Candidates should possess previous experience in immunology, developmental biology or reproductive physiology. Opportunities exist for a limited amount of teaching.

The post is available for up to 3 years in the first instance, with a salary on the scale £2,118 to £3,285, depending upon qualifications and experience, F.S.S.U. subject to accord of academic status. Starting date negotiable, but preferably within the next three months.

Applications (2 copies) should include full details of qualifications and experience, and the names of two referees, and be submitted to Dr. W. D. Billington, Department of Pathology, University Walk, Bristol BS8 1TD, as soon as possible. (1919)

#### UNIVERSITY COLLEGE CARDIFF

Applications are invited for the following post: RESEARCH ASSISTANT (Temporary) in the ZOOLOGY Department.

Salary Scale: £1,500 to £1,662. Duties to commence December 1, 1974 to March 31, 1976.

Applicants for the above post will be required to take part in a survey of faunal distribution in the Severn Estuary. The work involves collecting, using a light hovercraft, and laboratory analysis of samples. Applicants should be graduates with some zoological training and familiarity with two-stroke engines would be an advantage.

Applications, together with the names and addresses of two referees, should be forwarded to the Registrar, University College Cardiff, P.O. Box 78, Cardiff CF1 1XL by November 29, 1974. Please quote 0689. (1920)

#### UNIVERSITY OF BRISTOL DEPARTMENT OF PATHOLOGY

#### IMMUNOLOGY TECHNICIAN GRADE 4 OR 5

A vacancy exists within a research group investigating the immunological aspects of pregnancy for a Technician to take charge of the preparation and analysis of antisera and to participate in the various ongoing research projects. The person appointed will be the senior technician in the group, and will have responsibilities for the day-to-day running of the laboratories and the general supervision of other technical staff. Preference may be given to candidates with previous experience in column chromatography and electrophoresis, and ability to handle laboratory rodents is desirable. Initial salary on the scale £1,848 to £2,163 or £2,007 to £2,382, plus threshold payments under current arrangements (a revised scale is currently under review).

Applications with full details and the names of two referees to Dr. W. D. Billington, Department of Pathology, University Walk, Bristol BS8 ITD.

#### MERSEYSIDE COUNTY MUSEUMS

#### KEEPER (PHYSICAL SCIENCES) £3,573 to £3,939 p.a.

(plus threshold payment of £167.04)

Applications are invited for this post heading a new department concerned primarily with Astronomy, Space Technology and a Planetarium but which is expected to expand into other aspects of Physical Sciences.

Candidates should normally be science or engineering graduates with relevant research experience. Possession of the Diploma of the Museums Association would be an advantage.

Further particulars and application forms, returnable by November 29, 1974, are available from the Director (Dept 4), Merseyside County Museums, William Brown Street, Liverpool L3 8EN (Tel: 051-207 0001). (1939)

### SANDWELL AREA HEALTH AUTHORITY

#### **TECHNICIANS**

#### (MUSCULAR DYSTROPHY LABORATORY)

Two technicians with HNC or degree qualifica-tions are required for bio-chemical research into muscle disease. The post will provide opportunity to gain F.I.M.L.T. by thesis.

Salary and conditions of service according to qualifications as defined by Whitley Council.

qualifications as defined by whitey Council.

Applications stating age, qualifications, experience and naming two professional referees to the Hospital Secretary, Midland Centre for Neuro Surgery and Neurology, Holly Lane, Smethwick, Worley B67 7JX, to arrive by November 21, 1974.

#### UNIVERSITY OF OTAGO Dunedin, New Zealand LECTURER or SENIOR LECTURER IN ZOOLOGY AND MARINE BIOLOGY

Applications are invited for the position of Lecturer in Zoology and Marine Biology. This position is a joint appointment between the Department of Zoology and the Portobello Marine Laboratory, and the person appointed will be expected to contribute to the teaching programme of the Department of Zoology and to devote approximately half his time to research and other duties at the Marine Laboratory. Some preference may be shown towards candidates whose research interests are in marine field studies which utilise the resources of the sea-going research vessel of the Marine Laboratory.

Salary Scales—Lecturer: NZ\$7,361 to \$9,339 per annum; Senior Lecturer: NZ\$9,503 to \$12,142 with a bar at \$11,153 per annum.

Further information in the senior of the seni

Further information is available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University.

Applications close in New Zealand and London on December 15, 1974. (1935)

RESEARCH AND DEVELOPMENT WITH ROUSSEL

# **IMMUNOPHARMACOLOGIST** & PHARMACOLOGIST

We are looking for two people to strengthen a team working in the field of allergic diseases.

#### **IMMUNOPHARMACOLOGIST**

This position would suit a graduate or equivalent with some experience in immunopharmacology or related sciences who is looking for career development in this field.

#### **PHARMACOLOGIST**

This post would be of interest to a recent graduate in pharmacology or a related science who is interested in starting a career in research and development.

Roussel Laboratories Ltd is a progressive and expanding Company within a large international group involved in researching, developing, manufacturing and marketing a wide range of pharmaceutical and allied products.

Conditions of service and fringe benefits are exceptional. Assistance with relocation to this pleasant part of Wiltshire, with easy access to the surrounding countryside, will be

Please write with concise details of your qualifications and experience to

Miss R. Curtis, Personnel Officer, Roussel Laboratories Ltd.,

Covingham, Swindon, Wilts. SN3 5BZ



#### LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE (University of London) Keppel Street, WC1E 7HT POSTDOCTORAL IMMUNOLOGIST WINCHES FARM FIELD STATION, ST. ALBANS

Applications are invited for an appointment, which will be for three years initially, to work on a project on the immunology of schistosomiasis, in collaboration with parasitologists investigating vaccination against this disease. The successful candidate will be in charge of newly-equipped laboratories at St. Albans, and will be assisted by a schior technician. Initial salary within the range £2,118 to £3,108, depending on experience and qualifications, plus threshold payments.

Applications, consisting of curriculum vitae and naming two referees, should be sent to Dr. G. Webbe, at the School's London address, by December 6, 1974. Further information may be obtained from Dr. M. G. Taylor (St. Albans 53858).

#### EDITORIAL ASSISTANTS

Recent graduates in Microbiology or allied subject required to work on Microbiology Abstracts and Virology Abstracts. Duties include proof reading, abstracting and some editing. Please apply in writing to Dr E. S. Krudy, Information Retrieval Limited, 1 Falconberg Court, London WIV 5FG. Quoting Ref. No. (1925)

#### UNIVERSITY OF ADELAIDE WAITE AGRICULTURAL RESEARCH INSTITUTE WAITE PROFESSOR OF AGRONOMY

WAITE PROFESSOR OF AGRONOMY

Applications are invited for the above-mentioned appointment. The post was previously occupied, as the Waite Chair of Agriculture, by Professor C. M. Donald, F.A.A., who retired in March 1973. It has remained vacant pending the appointment of the new Director of the Institute, Professor J. P. Quirk, F.A.A.

The Professor will be Chairman of the Department of Agronomy for an initial period of three years, when the chairmanship will be reviewed, Applications are invited from persons active in the field of Agronomy or cognate disciplines. At present the major interests in the Department are in the fields of crop and pasture ecology and plant breeding. Information about the research and teaching of the Department of Agronomy is given in the Statement referred to below.

SALARY: SA19,614 a year with superannuation on the F.S.S.U. basis and, for an eligible candidate, supplementary superannuation and pension schemes.

FURTHER INFORMATION: Potential can-

clidate, supplementary superamacion and pension schemes.

FURTHER INFORMATION: Potential candidates are invited to obtain from the Secretary General, Association of Comomnwealth Universities (Appts), 36 Gordon Square, London WCIH OPF, or from the Registrar of the University, a Statement of Agronomy, the Waite Institute and the University, as well as the formal terms of appointment. Any further information desired about the conditions of appointment or the University generally should be sought from the Registrar; further information about research work, facilities, etc. in the Department or the Waite Institute may be sought from the Director of the Institute.

of the Institute.

APPLICATIONS (in duplicate) giving the particulars listed in paragraph 7 of the Statement should reach the Registrar of the University, G.P.O. Box 498, Adelaide, South Australia 5001, not later than January 31, 1975. (1936)

#### MASSEY UNIVERSITY Palmerston North, New Zealand LECTURER IN GENETICS

Applications are invited for the above-mentioned position in the Department of Microbiology and Genetics; the department is responsible for courses leading to the B.Sc., B.Sc.(Hons), M.Sc. and Ph.D. degrees. The successful applicant will assist in the continuing development of Genetics as an area of teaching and research and will participate in the offering of an introductory course in cell biology to first year students. Applicants who have experience in tissue culture studies and interests in cytogenetics and aspects of cell structure and function will be given favourable consideration.

Salary: NZS7,361 to NZ\$9,339.

Further details of this position and of the University, together with general conditions of appointment may be obtained from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF, or from the Registrar of the University.

Applications close on January 15, 1975.

#### UNIVERSITY OF TROMSØ NORTH NORWAY FISHERY BIOLOGY RESEARCH GROUP

Applications are invited for the following positions:

#### TWO PROFESSORSHIPS AND ONE LECTURESHIP

ONE PROFESSORSHIP in Fisheries Biology for candidates with appropriate qualifica-tions and experience in the fields of Population Dynamics and/or Systems modelling. Duties will include development of courses and teaching of fishery students in these topics plus research in an integrated programme of fjord fish populations and their

ONE PROFESSORSHIP AND ONE LECTURESHIP IN AQUACULTURE. There is much interest in aquacultural developments and possibilities in North Norway, in which the successful candidate will have ample opportunity to participate. It is expected that those who are appointed contribute to the fjord research programme. Though experience in aquacultural techniques is required it is also hoped that the persons appointed will have a broad biological experience so that in both teaching and research he will be able to stress the more fundamental aspects of this subject.

The University is now considering long-range plans for expansion of its teaching and research capability in Fisheries Biology. A major programme of fishery and environmental research of fjords is being developed by a group of biologists headed by Professor A. H. Weatherley. Other fishery research programmes are being planned.

weatheriey. Other inshery research programmes are being planned.

Tromso is situated in a very large northern commercial fishing region and fishery biology is one of the areas of study being emphasised in the development of the University. The work of the fishery group is being conducted in conjunction with marine biologists, and fishery biology will be offered at both undergraduate and graduate levels. English may be used for teaching until Norwegian is learned.

The University is new and expanding rapidly, and those who obtain posts will be expected to participate in planning activities. The fishery group is part of the Institute of Biology and Geology and is housed in a new building well equipped for various kinds of teaching and research.

A new highly sophisticated 112 foot fishery research useful will be qualible by 1075.

A new highly sophisticated 112 feet fishery research vessel will be available by 1975. The University has a computer centre featuring a 64-K Nox computer, there is a DCT 2000 Simulator connected to a UNIVAC 1110 at the University of Bergen, and DCT 1000 Simulator is under construction.

Tromsø lies north of the Arctic Circle (lat. 69°), but has a mild climate because of its coastal position. There are two months of midnight sun and two of darkness. There are fine opportunities for outdoor life.

Salaries: Professor 104.780, Lecturer 69.760 to 87.510 N. Kr. per year. The University will help with housing and other problems. Applications should be sent by December 12, 1974, including five copies of curriculum vitae and three sets of relevant publications.

Formal applications should be addressed to: Universitetet i Tromsø, Postboks 635, 9001 Tromsø, Norway. Further enquiries should be addressed to Professor A. H. Weatherley, same address. (1941)

#### CENTRE FOR OVERSEAS PEST RESEARCH Porton Down, Salisbury, Wilts

ASSISTANT SCIENTIFIC OFFICER

Routine maintenance of colonies of several insect pests of agricultural, veterinary and public health importance: moths, flies and mosquitoes. Bioassay of insecticides, viruses and other pest materials.

control materials.

Qualifications: Four "O" levels including English and a science subject.

Salary: £887 to £1,899, according to age.

Application forms from Miss M. B. McKiernan, Centre for Overseas Pest Research, College House, Wrights Lane, London W8 SSJ. Ref. COPR/3/4/64. Closing date: November 30, 1974.

#### UNIVERSITY OF RHODESIA DEPARTMENT OF AGRICULTURE LECTURESHIP/SENIOR LECTURESHIP IN CROP SCIENCE

Applications are invited from suitably qualified graduates for a post of Lecturer or Senior Lecturer in the field of Crop Science, which includes specialised treatment of crop physiology, pasture science and the production of field, forage and horticultural crops. The successful candidate will have, in addition to a basic knowledge of the botany of agriculturally important crops, research experience in one of these special areas.

Salary Scales (Approx. Stg. equiv.): Senior Lecturer: £5,597 by 124 to £7,389; Lecturer Grade 1: £5,087 by 188 to £5,839; Lecturer Grade 11: £3,135 by 161 to £3,618 by 179 to £3,976 by 188 to £4,728 by 179 to £4,907.

Family passages and allowance for transport of

Family passages and allowance for transport of effects on appointment. Installation loan of up to half of one year's salary if required. Unfurnished University accommodation guaranteed for a period of at least three years for persons recruited from outside Rhodesia. Sabbatical and triennial visits with travel allowance. Superannuation and medical aid schemes.

aid schemes.

Applications: (6 copies) giving full personal particulars (including full names, place and date of birth, etc.), qualifications, experience and publications, and naming three referees, should be submitted by December 15, 1974, to the Assistant Registrar (Science), University of Rhodesia, P.O. Box MP.167, Mount Pleasant, Salisbury, Rhodesia, from whom further particulars may be obtained, Applicants from outside Southern Africa should send a copy of their application to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from whom further particulars may also be obtained. (1927)

#### THE UNIVERSITY OF ASTON IN BIRMINGHAM

DEPARTMENT OF BIOLOGICAL SCIENCES

#### VISITING LECTURER

Applications are invited for a Visiting Lecturer in the Department of Biological Sciences, for one year, to assist in the teaching of environmental studies and to contribute to some aspect of the research in the department.

Salary will be within the range £2,118 to £2,580 per annum.

Requests for further details and application forms (which should be returned within 14 days of the appearance of this advertisement), should be sent, quoting Ref. No. 003/6 to Staff Officer, the University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET. (1928)

#### FELLOWSHIPS AND **STUDENTSHIPS**

### UNIVERSITY OF CALGARY

#### DEPARTMENT OF **PHYSICS**

Applications are invited from suitably qualified persons (M.Sc. or Ph.D.) for a position as Research Assistant/Postdoctoral Fellow in the field of experimental/observational AERONOMY. Interest and/or experience in near infrared atmospheric emissions considered advantageous.

Write for further information and giving resume of background to: Professor A. W. Harrison, Department of Physics, Calgary, Alberta, Canada, T2N 1N4. (1774)

### NATIONAL RESEARCH DEVELOPMENT COUNCIL

Applications are invited for two N.R.D.C. Fellowships. The successful candidates will work under the direction of Dr K. Jewers at the Tropical Products Institute on the isolation and transformation of biologically active compounds from tropical plants. The Fellowships are tenable for one year in the first instance with the possibility of renewal subject to satisfactory progress being made.

Applicants should have a degree or equivalent in chemistry plus at least two years' postgraduate experience.

Salary in the region of £2,689 per annum plus an annual leave allowance of 22 days.

### NRDC Research Assistantship

Applications are also invited for an N.R.D.C. Research Assistantship. The successful candidate will work under Dr K. Jewers at the Tropical Products Institute of the pharmacology of biologically active constituents isolated from tropical plants. This post is also tenable for one year in the first instance with the possibility of renewal for a further period subject to satisfactory progress being made.

Applicants should have a degree or equivalent in pharmacology. Salary £1,820 to £2,040.

Application forms for both these posts from Miss C. A. Hall, Tropical Products Institute, 127 Clerkenwell Road, London ECTR 5EB.

(1892)

#### UNIVERSITY OF BRISTOL

### JOINT RESEARCH PROJECT

for a period of three years, has been arranged between Plant Protection Limited, I.C.I. Jealott's Hill Research Station, and the Department of Physical Chemistry, University of Bristol, under the joint I.C.I. Fellowship scheme for work on the fundamental parameters which control the properties of concentrated colloidal dispersions.

Applications are invited for the post of post-doctoral fellow to carry out research on this project. The salary will be in the range £2,247 to £3,108 per annum, F.S.S.U.

Applications should be sent to Professor R. H. Ottewill, School of Chemistry, University of Bristol, Bristol BS8 1TS. (1856)

### UNIVERSITY OF STRATHCLYDE

Applications are invited from graduates in Physics, Chemistry, Chemical Physics or other relevant disciplines for an S.R.C. POSTDOCTORAL FELLOWSHIP in the DEPARTMENT OF CHEMISTRY.

The successful applicant will work on Energy Transfer Studies using the tunable SPIN FLIP RAMAN LASER in collaboration with Drs. R. T. Bailey and F. R. Cruickshank.

The post is for one year in the first instance with possibility of extension for one further year. Salary up to £2,247 per annum plus threshold payments, placing according to age and experience.

Applications (quoting R40/74) to Dr. R. T. Bailey, Department of Pure and Applied Chemistry, University of Strathelyde, Thomas Graham Building, 295 Cathedral Street, Glasgow G1 1XL. (1893)

#### UNIVERSITY OF NEW ENGLAND Armidale, New South Wales

#### RESEARCH FELLOW-AGRONOMY AND SOIL SCIENCE

A vacancy exists for a postdoctoral research fellow in the Department of Agronomy and Soil Science to work with Dr Graeme Blair on aspects of phosphorus turnover rates in pastures. Of particular interest is a study of factors regulating the pool size and turnover rate of organic phosphorus.

The appointee will be expected to develop his/ her own programme of research within the framework of ongoing field studies of phosphorus cycling in pastures grazed by sheep and cattle. This is a joint programme under study at Grafton and Tamworth in northern New South Wales and funded by the Australian Meat Research Committee

The Department of Agronomy and Soil Science has field, glasshouse and controlled environment facilities available as well as automated equipment for analytical and radioassay work.

The appointment will be for one year initially with possible extension to three years dependent upon satisfactory performance and availability of

Salary \$A9,002 to \$12,352.

Further information can be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.
Applications close on November 30, 1974.

### THE POLYTECHNIC CENTRAL LONDON

#### School of Engineering and Science Bio-Organic Research Group

Applications are invited for a Research Assistantship or a Research Fellowship (postdoctoral) for work on the entry of drugs into mammalian cells in culture. The successful candidate for the Research Assistantship will be expected to register for a higher degree.

Research Fellow £2,151 to £2,331 Research Assistant £1,544 to £1,654 Application form from: The Establishment Officer, PCL, 309 Regent Street, London W1R 8AL.

(AK1926)

#### FLINDERS UNIVERSITY OF SOUTH AUSTRALIA RESEARCH FELLOWSHIP IN BIOLOGY

RESEARCH FELLOWSHIP

IN BIOLOGY

Applications are invited from suitably qualified persons for a Research Fellowship tenable within any of the fields represented in the School of Biological Sciences of the University. The Research Fellowship is tenable initially for one year but application may be made for a further year. Applicants should have completed a Ph.D. degree prior to October 31, 1974, or have had equivalent research experience.

The appointment will be made according to qualifications and experience within the salary range \$A8,184 (3 by \$342) (1 by \$313) to \$A9,523. A grant not exceeding \$A500 may be made as contribution towards the travel costs of the Fellow in taking up the Fellowship.

The School of Biological Sciences is an integrated school, without sub-divisions into separate departments. Research conducted in the School includes the following fields of study: Behavioural Biology (drug tolerance, neurotransmitters and learning), Biochemistry (regulation of growth, carcinogenesis by polycyclic hydrocarbons, purine analogues, control of D.N.A. synthesis, chromatin sub-structure, cytokinins, effects of aldosterone and analgesics on kidney), Biophysics (energy conservation in photosynthesis, fast movements in plants, solute transport by plant glands, vision physiology and anatomy), Cytotaxonomy (of arid zone flora), Developmental Biology (germ plasma in Anura, reproductive embryology, nuclear transplantation). Genetics (population genetics, radiation and chemical mutagenesis, genetics of Drosophila compound chromosomes. Drosophila development), Immunology (mechanisms of self-tolerance, adjuvants, murine leukaemia viruses). Microbiology (control of viral macromolecular synthesis, viral pathogenesis, genetic control of recombination in Neurospora), Physiology (Animal) (locomotion, temperature and water homeostasis, responses of liver and abdominal nerve receptors to various stimuli, physiology and paleobiology of marsupials). Physiology (Plant) (structural and functional relationships between the plan

species).
Further details concerning the staff of the School and their fields of interest are obtainable from the Registrar, The Flinders University of South Australia. Budford Park, South Australia 5042.

Applications, including full personal details, details of academic record and publications, a brief outline of research interests and names of at least two referees, should be lodged with the Registrar by December 1, 1974. Each applicant should ask his or her referees to forward their reports directly to the Registrar before the closing date. (1865)

#### ALCOHOLISM M.C.A. RESEARCH FELLOWSHIP

The Council offers a Fellowship of 2 or 3 years to a medically or scientifically qualified worker for research into the cause, detection or prevention of alcohol dependence. The financial support will be adjusted to the experience of the worker and the demands of the proposed research. Full information from: The Executive Director, The Medical Council on Alcoholism, 8 Bourdon Street, London WIX 9HY. (1864)

#### TWO POST-DOCTORAL FELLOWSHIPS IN THE EARTH AND PLANETARY SCIENCES

The Lamont-Doherty Geological Observatory of Columbia University invites scientists interested in any field of the earth and planetary sciences to apply for the following Fellowships.

Two Post-Doctoral Fellowships, each awarded for a period of one year (extendable to two years in special instances) beginning in September, 1975, with a stipend of \$12,000 per annum. Completed applications are to be returned by February 15. Application forms may be obtained by writing to the Director, Lamont-Doherty Geological Observatory, Palisades, New York, 10964

Award announcements will be made March 31, 1975.

#### UNIVERSITY OF OTAGO Dunedin, New Zealand TEACHING FELLOW IN THE FACULTY OF SCIENCE

Applications are invited for a Teaching Fellowship available in Departments of the Faculty of Science. Duties will include teaching and research, the latter normally being directed towards the degree of Ph D degree of Ph.D.

Stipend: NZ\$3,913 per annum for two years. NZ\$4,160 for the third year.

Further particulars are available from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WCIH 0PF or from the Registrar of the University.

Applications close on November 30, 1974.

#### UNIVERSITY OF MELBOURNE RESEARCH FELLOWSHIPS

Several fellowships are awarded twice each year for full-time research in any department of the University.

Eligibility: Ph.D. or at least equivalent research experience

Tenure: 1-2 years.

Salary:

\$9,002 (Australian), (currently equivalent to U.S. \$13,368 or £5,761), \$485 annual increment, plus return air fare.

GRANTS-IN-AID AND TRAVEL GRANTS Several grants-in-aid are also awarded twice each year to academics on salaried leave to conduct full-time research at the University.

Detailed information from the Secretary for Graduate Studies, University of Melbourne, Parkville, Victoria, 3052, Australia or from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications close January 31, 1975. (1870)

#### UNIVERSITY OF SOUTHAMPTON DEPARTMENT OF AERONAUTICS AND ASTRONAUTICS

#### SENIOR RESEARCH FELLOW or RESEARCH FELLOW

Applications are invited for a Postdoctoral Fellow for research on the spray from motor vehicles. The research is part of a continuing programme strongly biased towards experiment and is aimed at improving the understanding of the basic mechanisms and pointing to practical solutions of the spray problem. Salary will be dependent on qualifications and experience and is within the range £2,100 to £4,000 per annum plus threshold payments and F.S.S.U. benefits.

Applications, including curriculum vitae and the names of two referees, should be sent to the Deputy Secretary's Section (Ext. 2400), The University, Southampton, SO9 5NH, quoting reference 323/R/NA. Closing date November 30, 1974.

#### GIRTON COLLEGE CAMBRIDGE

Applications are invited for a SCIENTIFIC RE-SEARCH FELLOWSHIP open to women graduates and tenable for three years from October 1, 1975. The applications should be in the field of Mathe-matics, Natural Sciences, Geography and allied

Each Fellowship is to the value of £1,000 per annum and pensionable. Particulars are available from the Secretary to the Council, Girton College, Cambridge, to whom applications should be sent by January 8, 1975.

#### UNIVERSITY OF STRATHCLYDE

Applications are invited from CELL BIOLOGISTS for a RESEARCH FELLOW-SHIP (Postdoctoral preferred) in the DEPARTMENT of PHARMACEUTICAL TECHNOLOGY starting as soon as possible, to work until December 31, 1976, on drug actions on cultured human cells.

actions on cultured human cells.

Salary range £2,118 to £2,412 plus threshold payments and F.S.S.U.

Applications (quoting R41/74) with curriculum vitae and names of two referees to Dr Mary Dawson, Department of Pharmaceutical Technology, University of Strathclyde, Royal College, 204 George Street, Glasgow G1 1XW, from whom further particulars may be obtained (Tel: 041 552 4400 Ext. 2113).

#### CRYOGENICS RESEARCH UNIVERSITY OF SOUTHAMPTON

#### POST-DOCTORAL RESEARCH FELLOWSHIP

Applications are invited for the following two year appointment to commence as soon as possible.

#### L.N.G. HEAT TRANSFER

To measure 2 phase condensing heat transfer coefficients on large rig at A.E.R.E., Harwell for L.N.G. heat exchangers.

Applicants should have experience in mechanical/chemical engineering or low temperature physics.

Salary in range £2,250 to £2,600 (plus F.S.S.U.)

Applications with names of two referees to the Deputy Secretary's Section, The University, Southampton SO9 5NH. Please quote reference 254/R/Na. (1912)

#### **GRANTS & SCHOLARSHIPS**

#### UNIVERSITY OF SYDNEY THE HENRY BERTIE AND FLORENCE MABEL GRITTON POSTGRADUATE RESEARCH SCHOLARSHIP

Applications are invited for the abovementioned Scholarship from graduates of any university within the Commonwealth, or from graduates of any university who are British subjects. The Scholarship, which is tenable in the University of Sydney, will be awarded in the first instance for one year, and may be renewed for a second year and, in exceptional cases, for a third year.

The scholar will be expected to undertake post-graduate research in Chemistry as it is related to Industry or Agriculture.

Depending on an applicant's qualifications, the Scholarship will be awarded in either the senior grade (in the range of \$A7,270 to \$A8,413 per annum) or the junior grade (stipend \$A3,550 per annum), plus other allowances where appropriate. At either grade, a travel allowance is provided.

Applications, which should include a certified copy of the applicant's academic record and the names of two referees, close with the Registrar, University of Sydney, N.S.W. 2006, Australia on January 17, 1975.

Further information and application forms may be obtained from the Registrar's office. (1871)

### £600 POST-GRADUATE **GRANTS**

### **MSc Dairy Science** at Reading University

The Dairy Industry offers a limited number of special grants worth £600, plus all fees, for the one-year MSc course in Dairy Science at Reading

This post-graduate course is available to holders of first degrees or equivalent qualifications in pure or applied science (including agriculture).

The awards are designed for those students who show outstanding potential for responsible scientific, technical and managerial positions in the processing and manufacturing sides of the Dairy Industry.

The MSc qualification could also lead to lectureships in dairy science and technology.

Applicants must be resident in the United Kingdom, and intent upon taking up employment in the United Kingdom Dairy Industry on completion of the course. All applications for the 1975 award must reach us by 31 January 1975.

For full details, please write to, or

R D Lemmer Esq., Department 11, The Dairy Industry Training & Education Committee, The Joint Committee, Giggs Hill Green, Thames Ditton, Surrey KT7 OEL. Tel:01-398 4101. Ext. 436.

(1887)

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#### LECTURES AND COURSES

#### UNIVERSITY OF LONDON:

A course of three lectures entitled "The Population Biology of Plants" will be delivered by Professor J. L. Harper (Bangor), at University College (Chemistry Auditorium), Gower Street, W.C.1, on November 26, 27 and 28 at 5.30 p.m. ADMISSION FREE, WITHOUT TICKET Academic Registrar (1904)

# **EUROPEAN MOLECULAR BIOLOGY ORGANISATION**

Postgraduate Course

in

# Advanced Animal Virus Genetics and Molecular Virology

An E.M.B.O. study course in Advanced Animal Virus Genetics and Molecular Virology will be held in the Institute of Virology, University of Glasgow, from March 16 to April 4, 1975 under the direction of Professor J. H. Subak-Sharpe. The intensive laboratory periods will be supplemented by 15 lectures as well as specialised discussion. In addition there will be 7 special guest lectures.

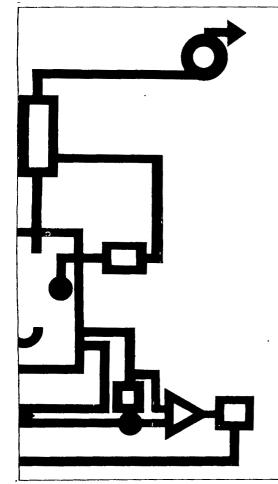
The Course will be particularly appropriate for research workers in the field with three or more years postgraduate research experience as the programme will cover more recently developed techniques used in the genetic and biochemical analysis of animal virus systems, for example, triparental cross recombinants, restriction enzyme fragment analysis, or application of enucleate cells in animal virology.

The fee is £90 excluding accommodation. The number of places is restricted to 16. Limited E.M.B.O. funds are available to help those applicants who could not otherwise attend.

For further details and application forms write to:

Professor J. H. Subak-Sharpe, The Course Director, The Institute of Virology, Church Street, Glasgow G11 5JR, Scotland. Closing date for applications is January 6, 1975. (1903)

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### **Gas Analysis Instrumentation**

Anthony Verdin

The detection and analysis of gases and vapours has become essential in many areas of modern life; noticeably industrial safety and control of the environment. As a result a whole class of physio-chemical methods has come into existence. The author draws on his fourteen years of experience in the field to supply the only wide-ranging view of all types of equipment presently available. Both academics and industrialists will find it indispensable.

### **Electrical Breakdown in Gases**

Edited by J. A. Rees

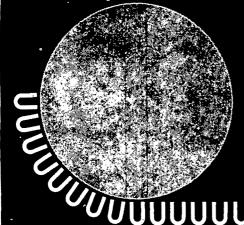
The electrical breakdown of gases has been studied in the laboratory since before the start of this century and it is sixty years since Sir J. S. Townsend wrote his monograph *The Theory and Ionisation of Gases by Collision.* In that time the subject has been widely studied and a great deal of progress has been made in understanding many aspects of the problem. J. A. Rees has selected the major papers in this field and has linked them with comments and an introduction. Reprinted from the the original sources, the papers together provide a unique introduction to the ionisation of gases.

# Introduction to Space Charge Electron Optics

G. A. Nagy and M. Szilagyi

This introduction to space charge electron optics is an updated translation of an original Hungarian publication which presents to the West for the first time a comprehensive survey of the vast work carried out in this field in the USSR and Eastern Europe. It is however more than just a summary of literature as it also contains the solution of many problems and so points the way to future developments.

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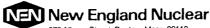
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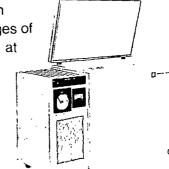


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- K. B. Sharpless and R. F. Lauer, J. Amer. Chem. Soc., 94, 7154 (1972).
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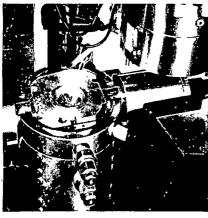
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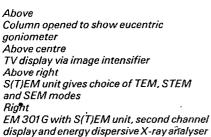
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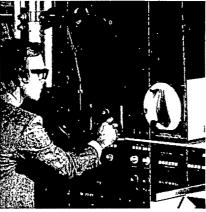
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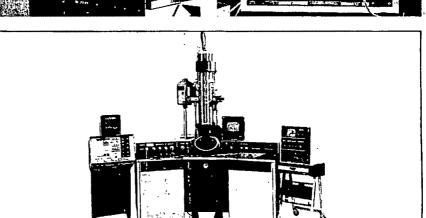
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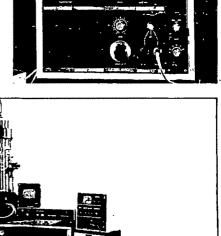
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#### **Cover Picture**

Sir John Tenniel's Red Queen in Through the Looking Glass.

Dr Van Valen's Red Queen hypothesis of evolution is described on page 298 and in News and Views on page 269.



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- Articles are up to 3,000 words in length with at most six displayed items (figures and tables) and may either be reports of major research developments in a subject or broader reviews of progress.
- Letters are brief reports of research of unusual and wide interest, not in general longer than 1,000 words; at most they have three or four displayed items (figures and tables).
- 'Matters Arising' permits occasional short discussion of papers that have previously appeared in *Nature*. A limit of 300 words is placed on contributions.

Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the Système International. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible, exp (a) is preferred to e<sup>a</sup> if 'a' is more than one character. Articles should be accompanied by an abstract of not more than fifty words, and the abstract should list the main conclusions that are drawn.

References are indicated by superscripts in the text. See any contemporary Nature for style, but note:

- (i) To refer to several references by the same author at once, only one reference number need be given.
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A fuller guide appeared in Nature (246, 238; 1973).

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November 22, 1974

# Academics in the boardroom

A FEW years ago a colleague working in a university was approached by an establishment of the Ministry of Defence with an invitation to become a consultant on some matters in which he was an expert. He underwent an extensive security grilling (the establishment had an unenviable record of leaks and was sensitive to recent criticism) and a year later was pronounced fit to be consulted. After the first few days at the site he returned to his university where several weeks later he was called by the head of the section for which he had consulted. "I'm terribly sorry to trouble you", he said, "but can you tell me whether you think your daily rate for consulting should be £4, £7 or £10?"

Is consultancy worth anything at all beyond those few pounds sterling either to industry or to universities at present? The question is touched lightly upon in The Universities and Applied Research, the proceedings of a day's symposium of industrialists and university scientists earlier this year, published by the Research and Development Society (47 Belgrave Square, London SW1; £4.50). The meeting was organised to look at the relevance of applied research in universities to social and industrial needs. One of the subjects which obviously came in for some attention was the ability of university staff to be responsive to industry—assuming they wished to—and there seemed to have been general agreement that consultancy was a good thing. Thus Professor G. V. R. Born (University of Cambridge) saw consultancy as "a fascinating assignment ... a less expensive way of providing industry with information and ideas [than taking similar persons on full-time]". And Professor K. Hoselitz (Mullard)—"university consutlants (were) a useful way of forming a bridge". And Dr A. Spinks (ICI) "strongly approved of consultants". Can one therefore be satisfied that the consultancy system is an adequate means of broadening the understandings between industry and university, or is there more that could be done in the creation of formal machinery for the movement of highly intelligent people?

Despite the good intentions expressed above, there are many who regard consultancy cynically. On the university side there are those who give very poor value for money, who refuse to see industry in any broader perspective than that of the immediate problem presented to them or who dump the work on their students as unpaid sub-consultants. There are those who arrange with colleagues to provide mutually contradictory advice so that they can profit by regular recapitulation of the same opinion to a confused managing director.

On the industrial side there are many who employ consultants simply because it is a prestigious thing to do. Others do not use consultants for ideas or criticism but to provide an academic rubber stamp for their activities and a reassurance that they are not making asses of themselves.

Obviously the value of a consultancy depends entirely on the calibre of the two parties to it in each case, but there seems to be a need in many instances to go beyond the rather flimsy structure that the arrangement has at present. One of the biggest difficulties of an ad hoc scheme is that the consultant is insufficiently tied to the organisation for which he consults. He is not brought far enough into the corporate decision making nor the day-to-day running to feel deeply identified with the need for the company to be profitable. He is also likely to be fed problems which the company believes he might be able to solve—and which the company may have gone to some trouble to concoct for him—rather than be invited to roam around and pick up the problem that seems to him most important.

Many of those in applied research and a few of those in pure research in universities have had some industrial experience, but the number has been declining in recent years. Much of this experience, however, is at the junior scientific and technical level. What is now needed is more opportunities for the process of consultancy to move towards one in which it would be natural for senior university staff to consider a spell of up to several years in industry at the boardroom level. There is no doubt that many academics would find the industrial challenge stimulating and many industries would welcome new blood at the executive level. In addition the still rather distant relationships between industry and university in Britain could not but be helped by this.

The biggest problems are those man-made difficulties of tenure and pensions. It is ridiculous the extent to which careers become controlled by these constraints. There is hope that the quality of some pension schemes and their interchangeability will improve in the next year or two. The problem of university appointments remains and is not easily solved. It needs some imaginative thinking.

### A hundred years ago



THE Journal of the Society of Arts states that M. Mège Mouriès, after analysing butter, has succeeded in making it synthetically. This imitation butter, recognised by the Conseil d'Hygiène as indistinguishable from real butter, is finding its way into the Paris markets at half the present price of real butter.

From Nature, 11, 76, November 26, 1874.

# For those in peril: 2

Charlie Clutterbuck, Alan Dalton and Andy Solandt, of the British Society for Social Responsibility in Science, consider what action needs to be taken to identify and regulate industrial health hazards.

Over the past two decades the petrochemical industry has expanded more rapidly than any other. It is estimated that thousands of new chemicals are introduced into the production processes of the industry each year. One such chemical is vinylchloride monomer (VCM), which is used in the production of one of the world's most important plastics-PVC (polyvinylchloride). Unlike most of the new chemicals introduced into the work environment. VCM has been fairly well studied and was thought to be non-toxic. From being a harmless' compound twelve months ago, VCM is now, however, being described as "the occupational hazard of the century" (William Lloyd, United States National Institute for Occupational Health and Safety.) What then might be the dangers of those countless other chemicals that have never been tested as health hazards?

Dr Epstein of Case Western Reserve University Medical School, a specialist on industrial carcinogens, asserts that "In the absence of pre-testing, the the worker himself or herself, is unwittingly used as an involuntary test subject, to whom data are not generally available, if indeed they are ever collected and analysed." (Regulatory Aspects of Occupational Carcinogens presented to an International Chemical Federation Conference in Geneva in October 1974.) As a result of this post hoc procedure the health and livelihood of people on the shop floor and in the monitoring labs of industry are in constant jeopardy.

What can the concerned scientist do to help improve this disgraceful situation? One possible answer was suggested by Peter J. Smith in Nature (October 18). He proposed that scientists should admit moral responsibility for taking a clear lead in seeing that the ill effects of science and technology are eliminated or at least mitigated." He then went on to suggest that the ways in which scientists could take this 'clear lead' was to do thorough research on health hazards and to ensure that the results of this research be made public. He realised that this work had to be supported by vigorous campaigning to acquire the necessary finances. He also recognised some of the difficulties in .

obtaining statistics on health hazards. "Of course, employers frequently attempt to justify their refusal to disclose vital information on the grounds of commercial secrecy—a ploy which is sometimes legitimate, sometimes not."

Unfortunately, however, he was not at all clear on where this information should go and on how it could be presented in order to achieve maximum impact. Clearly the usual procedure of merely publishing in academic journals would accomplish little. Perhaps, as Smith implies in his article, the information should go to a body of elite scientists which would have "an immense potential for influencing public and government opinion." And is it merely opinion that we want to change?

The British Society for Social Responsibility in Science (BSSRS) has been actively combatting some of the hazards of work over the past year. Although this by no means makes us 'experts' in occupational health, it has furnished us with enough experience to enable us to offer a fairly concrete and systematic alternative to Smith's proposals. Before we describe this alternative we describe a case history which is a typical example of the handling of health hazards in industry.

In 1961, a medical officer at a Dow Chemical plant in the United States, discovered that VCM was the probable cause of serious liver damage in a number of men in the plant. On the basis of this, Dow drastically reduced its TLV (threshold limit value or maximum 'safe' time-weighted average) for VCM to 50 p.p.m. This action was made public. But none of the other PVC manufacturers followed Dow's lead. We had to wait until one man, Earl Parkes, had taken B. F. Goodrich of Kentucky to court twice to obtain compensation for his liver damage before the knowledge of the dangers of VCM became widely publicised. (Earl Parkes and two other men at B. F. Goodrich subsequently died of the liver cancer angiosarcoma.) So far, one death due to angiosarcoma has been confirmed in Britain. Recently VCM has been associated with lung cancer, painful swelling of the joints of the hands and feet and degeneration of the central nervous system.

Now that the dangers of VCM are widely known, what is being done to protect people from exposure to it? In both the United Kingdom and the United States, industry has adopted emergency standards of 50 p.p.m., with a time-weighted average of 25 p.p.m. The Department of Employment has

set up a special committee to determine a code of practice for VCM; it is important that it reports soon. It has been deliberating for 6 months already and apparently it has not as yet even discussed the problem of a TLV.

Perhaps it will adopt the recent OSHA (Occupational Safety and Health Act in the United States) standard of 1 p.p.m. While this reduction is a welcome improvement however, it must be recognised that the TLV is only a small part of the problem. For even though a relatively low standard has now been set for VCM the OSHA has completely ignored a special committee's report on ways of enforcing this standard. Dr Epstein has made this comment on OSHA's recommendation: "However, the major key provisions of the Committee, including those endorsed by corporate scientists on the Committee, were disregarded by OSHA, presumably under strong industrial pressure." These included recommendations of the committee to ensure the effective implementation of the carcinogen standards by instituting sensitive environmental monitoring systems and a permit system. (Regulatory Aspects of Occupational Carcinogens presented to the ICF Conference, Geneva, 1974.) We can only hope that the new United Kingdom Commission on Health and Safety at Work will prove to be more stringent and independent in its approach to the problem.

It is too early for BSSRS to define clearly 'workers' science'. Even so, there are a number of general guidelines which we can propose at this time based on our own work on VCM hazards at a BP plant in Fort Talbot. These guidelines apply to any 'expert', whether technical, medical or scientific, working in the occupational health field. They also apply to those technical people working in industry who feel that there is a clear and immediate need for action on occupational health in their own industries.

• Try to work primarily with the men and women who are actually exposed to the health hazard. This does not mean that one should ignore other groups interested in occupational health. Clearly, both scientific and shop floor workers must seek the help and cooperation of management and government bodies interested in these problems. Management should be consulted and valuable information exchanged. The Factory Inspectorate and/or the Public Health Office should be approached. With the new powers invested in it by the Health and Safety at Work Act, the Factory Inspectorate

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may prove to be a valuable partner for any groups seriously working to reduce health hazards in industry.

Liaison with the Trades Union involved is of paramount importance. For it is they who have experience of negotiating with management and they who have at their disposal, the services of the independent Centenary Institute of Occupational Health which specialises in the chemical analysis and medical evaluation of questionable materials.

• Remember that your main reason for being on the 'shop' floor is to exchange information and experience. The people on the shop floor can give you first hand knowledge of the processes they work with and the hazards that these processes involve. They can tell you how their plant/office actually works, not just how it is supposed to work. For your part you can help these men and women to understand the technical/medical explanation of the hazard and how to monitor and keep

the necessary records of the hazard.

- Unfortunately, even the introduction of reasonable health conditions is an issue which often involves conflicts with employers. Recently 5,000 men and women at the Shell/Chevron plant in California had to strike for 5 months just to have seven medical provisions written into their contract. This was done in the light of the fact that all the other major petrochemical companies in the United States had already agreed to medical provisions being written into their contracts. The scientist must be prepared to stand up in defence of working people. This may mean pressing their case in joint management/union committees or even standing up in court to put the facts as they see them. Two men in the United States, Dr Selikoff of Mount Sinai Hospital in New York and Dr Epstein, have actually done this.
- Be prepared to use the press and television to publicise your case. The BSSRS helped World in Action to do

the first exposé of the VCM problem in this country. Later we devoted 15 minutes of an Open Door programme (BBC2) to a discussion of the VCM issue.

• In cooperation with other scientific, production line, and office workers, press for the implementation by the government, management and trade unions of systematic pre-testing of industrial processes and materials for possible health hazards.

Clearly it is not enough for individual scientists to become involved in isolated local health hazard issues. There must be a coordinated body for scientists participating in the field of occupational health. This is precisely the role that the BSSRS has begun to play and hopes to develop systematically and comprehensively in the future. We cannot afford to finance teams of investigation or the much needed thorough long term research in this area. We hope, however, to be able to serve as a vital catalysing agent.

# international news

MINISTERS from 24 countries, including Britain's Denis Howell, met in Paris last week at the first meeting of Environment Ministers convened by the Organisation for Economic Cooperation and Development (OECD). The ministers met to discuss several recommendations put forward by the environment group of the OECD on the formulation of rational and coherent environment policies throughout the OECD countries.

One of the concepts discussed at the recent meeting was in fact accepted in outline in 1972, namely the Polluter Pays Principle', which in effect means limiting state aid for pollution control to industry while making it conform to certain standards. This will then mean that goods made by polluting industry will be more expensive, as they have to pay for their own pollution control. The consumer, it is argued, will therefore prefer the cheaper goods made by non-polluting industry. Some countries have already incorporated the principle in their legislation, although, exceptionally, state aid may be allowed.

The ministers also discussed the OECD's proposed code of conduct for dealing with the problem of transfrontier pollution. One of the main points of the code is that pollution exported to other countries must not exceed the levels permitted within the polluting country.

The most widely discussed instance

# OECD urges environment policies

by Eleanor Lawrence

of this type of pollution, as far as Britain is concerned, is the sulphur dioxide from Britain and from continental Europe which ends up over Scandinavia as acid rain, with dire environmental consequences. The OECD is at present in the middle of an international survey of air pollutionthe first such survey to use completely standardised monitoring methodswhich should clarify this situation. Commenting after the meeting, Mr Howell said that Britain might have to reconsider her power-generating policy if the results of the survey showed that sulphur dioxide emissions from power stations were the 'culprit'.

The problem of trans-frontier pollution also raises the question of whether people affected have the same rights in the polluting countries' courts as would citizens of those countries in the same situation. But unfortunately, even the citizens of some countries find it extremely difficult to gain standing on matters of pollution in the courts, so that this might prove to be an empty privilege.

Complications and inequalities could also arise because of the fundamental difference between the judicial structures in the various countries—Europe by and large follows Roman law whereas in Britain there is the added complication of being able to bring prosecutions under common law, which operates on a precedent system rather than on the basis of published statutes.

There is also the problem of who exactly is liable to prosecution. If treaties for lower pollution had actually been signed between polluted and polluting countries, the defendant might well be the Government in the person of the Secretary of State for the Environment. Other problems would, of course, be the usual ones which bedevil pollution legislation generally, such as collecting sufficient data to make a case which will be accepted in the courts in the first place.

At present, if an article manufactured in Britain is sent abroad and proves to be harmful, it may well be the distributors in that country who are prosecuted and not the manufacturing company. This has happened in Australia in the case of thalidomide, marketed there by a subsidiary of Distillers Company Ltd.

The effects of pollution will be far less easy to define in practice than even the effects of a manufactured article, and there is the prospect of a real field day for the lawyers.

"Nothing has happened to climate in the past few years that is not the sort of thing that has happened already in the past few centuries". These words from Dr J. S. Sawyer (pictured right) of the UK Meteorological Office, sum up the 'establishment' view of the present concern about climatic doom (see the review on page 335). Serious droughts and floods are part of the climatic pattern, and there seems to be nothing unusual about the present fluctuations. A crash programme of research, says Sawyer, is not the answer to the problems posed by such floods and droughts; there is a need for more people to be active in climatic research, but there is also a need for time for them to grasp the problem as a whole.

value of frequent claims that man's activities are affecting the atmosphere. nowhere near enough people to follow ceremonies and woe betide those who surface of the sea. up all the suggestions with detailed cannot deliver at the right time. It was And since exponential growth will have ment of Genetics.



Round Britain

ther extrapolation is fruitless.

On March 8, 1974, Mr U. Geller, who needs no introduction, demonstrated his metal-bending powers on Channel 79, Toronto. The two keys he selected from a large collection were the key to the Genetics Department and a Fellow's key to Trinity, number 13 "registered as issued to me personally". The genetics key was indubitably bent through 15°, though by what process we decline to speculate. Trinity's key remained unbent. Neutral observers will marvel not at Mr Geller's powers but at Dr Owen's ability to evade the Cambridge key audit for so many years.

• In view of the recent accident in Bantry Bay on the west coast of Eire, Sawyer is also dubious about the used up all the fossil fuel by then, fur- the development of the Vikoma system for the recovery of spilt oil by BP will be hailed as a guiding light for the He likens the situation to a game in • Devotees of the Oxbridge scene will petroleum industry. This system inwhich people vie to think up new pos- know of the enormous significance corporates an inflatable boom for consible manmade effects, and points out attached to keys. College keys are care-taining oil (mainly crude) and a that this is rather futile since there are fully accounted for in annual medieval skimmer for recovering it from the

Primarily designed for BP's own studies. He accepts at least one aspect with some alarm, then, that we read spills, the system has now become of man's effect on the atmosphere— in New Horizons, a Canadian journal commercially available. A towing vessel CO<sub>2</sub> levels are going up as fossil fuel of 'frontier' research, of the fate of costs £24,000, and the recovery system is burnt. With exponential growth, that two such keys belonging to Dr A. R. G. £22,000. Although under development will produce a rise in temperature by a Owen, now editor of that journal and since 1967 and already in widespread "just appreciable fraction of a degree at one time Fellow of Trinity College use, the system has assumed import-Celsius" by the end of this century. Cambridge and Lecturer in the Depart- ance as a result of future North Sea oil production.

### Arms and the common man

from Wendy Barnaby

A RECENT meeting in Lucerne left a distinct impression that if everything were left to expents-no matter how well intentioned—laboratories would be thriving while the lot of the common man would steadily deteriorate. The meeting, one of a series of conferences being held to update the 1949 Geneva Conventions, was called by the International Committee of the Red Cross to consider the prohibition or restriction of conventional weapons "that may cause unnecessary suffering or have indiscriminate effects". It was hoped that the discussions would pave the way for recommendations to the politicians, whose next conference will be from February to April 1975. Progress was, however, very slow.

Take the case of napalm. The meeting spent some time discussing whether it is an 'all-or-nothing' weapon: that is, whether a person escapes it altogether or is killed by no matter how small an amount he encounters. Statistics of deaths of American soldiers in Vietnam after napalm had accidentally been

dropped on them were quoted to prove that it is not such a weapon. Only four out of 53 men died. But the effectiveness of napalm against personnel varies greatly with the training, experience and equipment of the victims, and with the standard of medical treatment and the rapidity with which it is given. It is therefore spurious to use this figure as a basis for calculating the effects of drops on civilians. Soldiers are trained, equipped and often experienced-and these particular ones received all possible modern medical care 10-20 minutes after the accident. Small comfort for the peasants.

This all-or-nothing sort of argument only confuses the issue by inviting conflicting evidence and adding to the general confusion of the debate. After all, everyone agreed that severe burn wounds are probably the worst possible type of wound. Surely that recognition is more important than a precise determination of how much of the worst possible type of wound is necessary to kill. If military action is aimed at incapacitating the enemy, it is obviously inhumane to achieve this through severe burning when other, less traumatic means are available.

Many argued that the accuracy with which napalm can be delivered makes

its use more discriminate and therefore more humane than less accurate, high explosive and fragmentation weapons. A typical 100-gallon container scatters napalm over a reasonably clearly defined area of about one-quarter of a hectare, whereas artillery of typical accuracy has a circular error probable (the radius of the circle whose centre is the target within which half of the projectiles aimed at the target fall) of 10-20 metres and a range error probable of 30-60 metres. On this basis, napalm was said to have great military value in close air support operations, where it is desired to destroy specific targets near friendly troops. This argument would be all very well if we could be certain that napalm would only be used against structural targets and never against people. Unfortunately such certainty is impossible in any war. The military attractiveness of its accuracy cannot compensate for the risks taken with human suffering.

Similarly, the fact that the experts disagreed on the seriousness of wounds caused by high velocity bullets should not be allowed to paralyse efforts to restrict their use. The problem is urgent because European small-arms manufacturers have developed 5.56 mm guns capable of firing such bullets, which

they are hoping to sell to NATO countries to replace NATO's present 7.62 mm weapons. (In fact it was rumoured at the meeting that Denmark was waiting to hear the outcome of the discussions there before deciding whether to buy just such a new rifle). But again, the evidence was conflicting.

Dr B. Rybeck, the most senior medical officer in the Swedish navy, has carried out experiments on anaesthetised pigs in which he found that high velocity bullets could in general be distinguished from low velocity ones on inspection of the wounds caused by each. In other experiments in Sweden, anaesthetised dogs were shot with metal spheres in order to dissociate the usual tumbling effect of high velocity bullets from their other wounding effects. Blood from a dog which had been shot with a sphere was transfused into a second dog. When the impact velocity of the sphere was 1,000 metres per second the recipient dog suffered severe changes of regional blood flow after the transfusion—an effect not observed when the impact velocity of the sphere was 500 metres per second. Lieutenant Colonel Dr Robert Scott, of the Royal Army Medical Corps, has shot small calibre, high velocity bullets into blocks of gelatin and reported that these did in general produce a greater effect than 7.62 mm bullets, but only at short ranges. If the flight of the bullet into the gelatin was unimpeded, those of high velocity and low calibre deposited more of their kinetic energy in the gelatin than those of larger calibre and lower velocity; however, this was reversed if the bullets passed through a thin steel plate (to simulate the body of a truck, for example, under battle conditions) before entering the gelatin.

The politics of prohibiting the use of a shiny new gun, just when various countries are considering adding it to their armouries, are formidable. And yet there are hopeful signs. In the discussion on napalm, for example, the experts explicitly recognised that the politicians will have to take public opinion into account in determining whether it will be banned. It was even argued that the pressure of public conscience provided grounds for making the use of napalm illegal. So the common man does have a part to play. And he may well argue that he is more interested in establishing the principle of restriction of the use of various weapons, even if some doubtful cases do escape the net, than in allowing the discussions to bog down in technicalities while more countries acquire more harmful arms. In the case of the rifles, even the experts agreed that, in general, wounds caused by low velocity weapons such as pistols, carbines or

submachine guns are much less severe than those caused by rifles. If, on the basis of understandings as common as this, the public were to put enough pressure on the politicians, the experts could be forced to come up with some sort of restricting arrangement. But if the experts are left to devise categorisations of inhumane weapons to present to the politicians as a basis for action, the issues will simply be referred to more laboratories for more experiments the results of which will cause more disagreement. And in the meantime, as inhumane weapons spread to more countries, the danger to the common man will only increase.

# Van Wyk de Vries commission

from Graham Baker, Johannesburg.

South African universities for the most part enjoy considerable freedom of action in conducting their affairs, subject to the restriction of the country's race laws and in spite of some profound differences of opinion over political legislation between the government and the English-medium institutions in particular. The present undergraduate population of over 80,000 is growing at almost 10% a year and the universities are well aware of their obligation to make up for South Africa's acute shortage of skilled manpower, especially in the sciences and engineering. Furthermore, the government has of late become especially sensitive to the possibility that some universities might have become bases for political activity of which it disapproves.

It is therefore understandable that the academic community in South Africa has been eargerly awaiting the findings of a government-appointed commission of enquiry into white universities, which was tabled in Parliament on October 30. Under the chairmanship of Mr Justice J. van Wyk de Vries, the commission was set up in 1968, principally to consider the financing of universities but also to take a look at "student relations". The recommendations with regard to financing have been openly welcomed as giving the necessary boost for needed expansion. Thus, the commission proposes that up to 85% of running costs should be subsidised by the state and that existing and future capital debts should also be borne by government grants. In return, universities should pay an annual levy of R50 per student to the government. It has been noted that particular advantages from this will accrue to the smaller, more recently established institutions, which tend to be Afrikaans-medium campuses. It also means that universities

can only develop to an extent permitted by the amount of financial assistance provided by the state. Even so, these recommendations, if accepted by the government, are unlikely to come into effect before 1976 at the earliest.

The main sobjections to the report have been voiced in respect of its views on the non-financial aspects of university life, in particular the extent to which universities may be permitted to engage in political activity. The majority view of the commission is that if a university is politically active in a way the government sees as being 'irregular' and therefore outside its proper function, it will forfeit financial support from the state. Some vice-chancellors see in this a threat that universities must toe the ideological line of the government in power or suffer crippling consequences. They take this as interference with autonomy of the universities and, more bluntly, they see it as an attack on the English-medium universities in South Africa. The Afrikanns-medium institutions, in contrast, see an alignment with government policy as part of their social and political obligation.

Professor G. R. Bozzoli, Principal of the University of the Witwatersrand and a member of the commission, has spoken out strongly against this aspect of the report, as well as another, the 'finding' that South African universities were founded on a social order based on the principal of multinational separate development. As Professor Bozzoli points out, apartheid was only forced on the universities as a result of the Universities Act of 1959.

In a similar light, the commission rejects the view that universities should be free to make appointments without regard to race, colour or creed, and sees no merit in student bodies having representation on university councils and senates.

It happens that the Van Wyk de Vries Commission tabled its report only a few days before the Prime Minister, Mr Vorster, declared in a report-back meeting to his Nigel constituency that he needs just six months to bring about major changes in South Africa's social order, presumably a reference to legislation on race relations. The same message was also spelled out last week by some of Mr Vorster's colleagues at a congress of the Nationalist Party, doubtless a direct consequence of recent events at the United Nations and in neighbouring African states. Whether any moderation of government policy on apartheid will take the heat off the universities and permit any number of black students to be admitted once again to the white institutions, as some vice-chancellors would wish, remains to be seen.

ment in academic circles in the Soviet testify against five members of his that time, the World Congress of Psy-Union has led, over the past few years, group in whose possession incriminat- chiatrists was taking place in Mexico. to an extremely disturbing reaction ing (dissident) literature was found. from the authorities—the incarceration of dissidents in mental institutions. The chiatric as opposed to standard legal that Mr Fainberg attributes the fact case of Zhores Medvedev in 1970 is measures seem somewhat arbitrary. Of that Bukovskii was returned by the perhaps the best known, but Med- the five members of the Leningrad Serbskii psychiatrists to the court. vedev's rapid release within a month group in question, three (including the system of the Soviet Union and unable sent to penal psychiatric institutions. world scientific community in Medvedev's support, may well expect a considerable stay in an entirely inappropriate establishment.

One such long term internee, Viktor Fainberg, who was released in November 1973 and, after a brief reconfinement in April-May 1974, was finally allowed to emigrate to Israel, visited said that psychiatric procedures seem vide considerable background material offence in question carries a fairly mild concerning this abuse of psychiatry. The picture he gives is a horrifying one —of institutions in which the 'doctors' are officers of the MVD, wearing uniforms and bearing military ranks and titles; where convicted criminals serve out their terms in the capacity of male where the treatment facilities for somatic illnesses are scanty (Mr Fainberg himself, who developed thyroan endocrinologist only twice a year); under which such dissidents are comand where the death rate from the occasional routine operation such as appendicectomy is abnormally high because of inadequate post-operative basis of such background evidence. If care. Far more alarming in its implica- a dissident happens to be the child of diagnosis and the punitive use of drugs will be sent to a psychiatrist who is -a picture already publicised in 1973 particularly interested in that field. by Academician Andrei Sakharov, but from the descriptions of Viktor Fain-berg, who has actually been a candidate for such 'treatment'.

patient reaches such an institution may vary. Zhores Medvedev was seized without warning. The chemist Anatolii Chinov, caught trying to cross the only exceptions have been in response Soviet-Czechoslovak border in Decem- to world opinion, as in the Medvedev ber 1968, himself put forward a plea of insanity, on the advice of his cousin, Bukovskii, a former biology student a psychiatrist, who erroneously thought who was expelled from his institute that this offered him the best chance before graduation, has had a long hisof rapid release. (This was at the begin- tory of dissidence and had already ning of the new policy). Mr Fainberg spent some time in a penal psychiatric himself, who was a member of a small institution when, in 1971, he was sidents cannot be explained as a sincere human rights group in Leningrad arrested for publicising the cases of attempt, however misguided, at the which was in contact with the better General Grigorenko and of Mr Fain- therapy of persons genuinely regarded known Moscow group of Sakharov, was berg himself. Bukovskii was sent by as deviant from the social norm, but is sent for a psychiatric report after one the Court to the Serbskii Forensic Psy-rather a deliberate and cynical abuse warning. In his case, the offence was chiatric Institute in Moscow for obser- of professional skill.

### Soviet abuse of psychiatry

from Vera Rich, London

maximum sentence under the criminal code (six months of obligatory work, for anxiety or some emotional problem -may be used to substantiate the mitted. Indeed, says Mr Fainberg, the psychiatrist who is to report on a given dissident is sometimes selected on the

Asked if any of the detainees rereturned to the KGB certified sane, Mr Fainberg observed that in such cases The route by which a prospective is expected of them" and that if, on rare occasions, someone in the provinces does file a negative report, a abandoned. second opinion will be sought. The case or that of Vladimir Boukovskii.

The rise of the human rights move- that of contempt of court—refusal to vation but it so happened that, just at It is to Soviet fears of the possible re-The criteria of selection for psy-action of the assembled psychiatrists

Once in the penal mental hospital, was an exception, and the average dis- chemist Lev Kvachevskii) received says Mr Fainberg, the dissidents are sident, caught in the penal psychiatric terms in labour camps and two were generally isolated from other patients. Conditions are harsh (one hour of to reap the benefits of a pressure cam- Asked if he could suggest any possible exercise per day, permission to write paign such as that launched by the basis for the division, Mr Fainberg to relatives only once a fortnight, and then under supervision). 'Treatment' consists largely of massive doses of aminazine (chloropromazine) or haloperidol, far in excess of any legitimate therapeutic dose, given orally if possible or else by injection. Patients who refuse the tablets, he says, are beaten and kicked by the male nurses-injuries being officially attributed to the London recently. He was able to pro- to be particularly favoured if the patient "falling and hurting himself". The only way to refuse such compulsory medication is by the threat of hunger strike, since this, in the case of that is, forfeiture of a percentage of the more notable dissidents, can attract salary in his own case), whereas a undesirable publicity. Fainberg says 'patient' committed for compulsory that the remarks of certain of the staff psychiatric treatment may be held for indicated that they do not accept the an indefinite length of time. It would official diagnosis, and that the dissidents nurses, frequently terrorising the seem, too, that any background of themselves counter all attempts to conpatients and robbing their food parcels; mild psychological illness—of having in vince them of their own insanity by the past consulted a psychiatrist, even speaking only to those members of the staff who do not consider them mad.

The pattern of treatment of distoxicosis during his internment, saw standard diagnosis of schizophrenia sident patients varies, it would seem, throughout the Soviet Union, being most severe in outlying areas. The Dnepropetrovsk institution, where the unfortunate Chinov was subjected to 30 insulin shocks and to electroconvulsive therapy (ECT) before his relations, however, is the picture of false a broken marriage, for example, he tives managed to get him transferred to Leningrad, has a particularly bad reputation. In 1972, it was proposed to disperse all the dissidents from Moswhich emerges in far greater detail ferred for a psychiatric report are ever cow and Leningrad to institutions in remote provinces—a policy which led Viktor Fainberg to embark on a "experienced psychiatrists know what hunger strike (his fourth) and to smuggle out an appeal to Kurt Waldheim. This dispersal policy was then

> Soviet psychiatric theory, based as it is on Pavlovian behaviourist ideas, is peculiarly amenable to the concept that dissidents can be turned by psychiatric measures back onto the paths of correct Marxist-Leninist thought. But it would seem from Mr Fainberg that the Soviet use of psychiatry as a means of dealing with dis-

# correspondence

#### Moon matters

SIR,—If your editorial "Bringing men to the Moon" (October 25) had been that of any journal other than *Nature*, we should have ignored it. In view of your prestige and of your great influence within the scientific community, we have decided to answer it.

You have sought to discredit this conference on a number of counts. You write, in reference to a previous conference, "while the scientists thought it silly, the philosophers took it all very seniously". In fact, at this meeting, only two of the 20 scholars were philosophers.

The purpose of the International Conference on the Unity of the Sciences is to further the field of communication between science and philosophy. The response of scientists to matters are naturally different from that of the philosophers. This indicates ignorance of modern philosophy.

Your sarcastic reference to the Second Conference in Tokyo is most surprising, since the conference itself was a success. Proceedings are available to those interested.

As to the 'messages of congratulation' by Nobel Laureates—messages of good-will, you must admit, can only be sent by the free will of those who send them. The manner of your reference to this indicates a prejudice. To write in this vein is nothing less than to despise a person, whoever he may be.

You attribute the most sinister possible motives to the founder of the organisation under whose auspices this conference is taking place. Surely Mr Moon, as any other philosopher of science living in the free world, is quite free to be devoted to any philosophy that he may choose for himself. Would it be unnatural to think that the initiator of the International Conference holds a specific point of view.

The aims, purposes and the nature of the Foundation, as admirable as they are, as set forth in the conference brochure, were clear and precise. Unfortunately, however, your statements about it seem to suggest that you had never read it.

You say that not all the advisers were very pleased to have their names used in this way. Basically, we have no intention of using the names of eminent scholars for purposes contrary to their own wishes. As you may know one person who agreed early on to

serve as an adviser subsequently chose to withdraw for his own reasons. As to our other advisers, both in the United Kingdom and internationally, they contributed greatly to the preparations of this significant event.

A feature of your editorial, unfortunately, is that general statements, even parts based on objective fact, demonstrate the prejudice and the apparent ill-intentions of the writer.

The list of participants you refer to was printed several months ago and included several names among which were firm acceptances as well as tentative ones. You have used this document to make allegations of improper conference management.

In reply to your enquiry: as founder of the Foundation under whose auspices this conference is taking place, Mr Moon has a right to be present and, contrary to your suggestion, he will, I understand, actually deliver the Founder's address.

It is suggested that in future you avail yourself of a more rigorous methodology for the purpose of discrediting those scientific enterprises of which you disapprove.

Morals are but an attempt to examine goals. And how can any behaviour be judged scientifically except in terms of its ability to achieve a goal? Any other criterion you must admit can only be purely subjective.

It may be true that there is, at present, no scientific methodology for the examination of basic goals, but the fault is surely with modern science for not having developed it. For until it is developed, scientists must accept their incapacity to examine the very assumptions on which their work is based. They must content themselves with doing things, without ever knowing why they should be done.

Contrary to what you intimate, we have no ideological bias. A number of scientists from the Communist countries were invited to this conference but, as it happened, could not attend.

It is suggested by way of conclusion that you ought to have taken the trouble to meet the person of central responsibility for the activity in question rather than questioning a subordinate employee whose knowledge may well be limited and even inaccurate.

In this case, I encourage you to accept the invitation extended to you in June this year, and to attend the Third International Conference on the

Unity of the Sciences, in order that you may evaluate its true value and import first hand, rather than relying on second-hand and perhaps unreliable sources.

In your allusion to 'their Boeing complex and their propensity to amateur philosophising', in reference to the eminent scientists, you have intentionally or unintentionally demonstrated your contempt and disrespect for those "deeply concerned scholars" who are willing to contribute to the urgent issues of the unity of science and the relation between science and morals.

P. BRIAN WIJERATNE ICUS, London SW1, UK

The 'subordinate employee whose knowledge may well be limited and even inaccurate' was the 'Chief Public Relations Officer.'—ED.

#### Chemicals and cancer

SIR,—Your October 11 issue summarised some of the issues in the Aldrin and Dieldrin Suspension Hearing.

I was attributed-incorrectly-with five criteria which must be met before there is sufficient evidence of human carcinogenicity. These criteria appeared in the Administrator's Opinion of October 1, and are not quoted from any statement made by me. An EPA attorney and an EPA witness caricatured my opinions by suggesting that I would only recommend the removal of a product from the market after cases of human cancer had occurred. This is not-and never has been-my opinion. To avoid any misunderstanding I submitted an additional statement on this topic to the hearing record on September 11, in which I also indicated that their belief was apparently based on my answer to the question whether we would take dieldrin off the market if human cancer cases occurred.

My affirmative answer did not imply that I would not give advice to that effect at an earlier stage. It is not my position that we must wait for human cancer before removing a product from the market.

I was, therefore, surprised to see in the Administrator's Opinion that my "demands are practically impossible to meet".

D. E. STEVENSON

Tunstall Låboratory, Sittingbourne, Kent, UK

# news and views

# Vaccination against malaria

ATTEMPTS to find a vaccine against malaria began in the 1930s but gave way to searches for new drugs during the war and anti-mosquito programmes after it. The combined use of anti-malaria drugs and insecticides has been one of the success stories of this century. The number of people infected with malaria has decreased considerably over the past decade in spite of the fact that, because of the increasing world population, more people than ever before now live in malarious areas. But resistance to drugs and insecticides, lack of money and political instability have reduced the future prospects of malaria eradication and thoughts have again turned to the possibility of developing a successful vaccine.

Three different sorts of vaccine are at present being investigated: irradiated sporozoites from the mosquito, extracts from schizonts (developing stages in the blood) and emulsified merozoites (the stages which pass between blood cells). After promising results using irradiated sporozoites in rodents, an experiment with Plasmodium falciparum in humans has resulted in one of three volunteers being protected against homologous (Clyde et al., Am. J. Med. Sci., 226, 169; 1973) and heterologous strains of the same parasite (Clyde et al., Am. J. Med. Sci., 266, 398; 1974). Earlier this year Simpson, Schenkel and Silverman (Nature, 247, 304; 1974) succeeded in protecting rhesus monkeys against Plasmodium knowlesi using non-viable fractions extracted from disintegrated parasites. This and earlier studies, taken together, have shown that eleven out of seventeen vaccinated monkeys survived challenge whereas eight controls did not. Both these kinds of approach obviously have some potential promise but they still need a considerable amount of development.

The most promising vaccination results so far achieved with monkeys are reported in this issue of Nature by Mitchell, Butcher and Cohen from Guy's Hospital Medical School. They used Plasmodium knowlesi and their vaccine consisted of emulsified cultured merozoites. Six monkeys were immunised in various ways and challenged some time later with the homologous parasite or heterologous variants. Protection against the homologous parasite was absolute in two monkeys and strong in the third. There was also considerable protection against heterologous variants and the parasitaemias never rose above 1.5% whereas control animals died after about a week. Subsequent challenges with other variants resulted in equally encouraging results. The merozoite vaccine was found to be species specific and afforded only minimal protection against challenge with Plasmodium cynomolgi bastianellii.

These results show that the merozoite vaccine induces immunity better than that obtained after repeated challenges and cures. This contradicts the belief that vaccination against malaria cannot induce an immunity better than that which can be acquired naturally. The fact that the immunity induced transcends challenge with several variants suggests that the antigenic variation that occurs in malaria is not an insuperable barrier to vaccination, as has already been shown by Clyde and his co-workers. Of particular interest is the fact that the merozoite vaccine is simple to prepare and that 1 ml of parasitised blood cells provides twenty

immunising doses. These are early days yet and the next step will have to be the evaluation of the merozoite vaccine in owl monkeys, which are the only suitable primates that can be infected with human malaria. These monkeys are becoming increasingly difficult to obtain as thousands have been used for testing drugs. Very soon malariologists will have to draw up priorities for the use of owl monkeys and the whole drug/vaccine argument will have to be thrashed out afresh.

F. E. G. Cox

# Messenger RNA doing without poly(A)

One of the tenets of cell biology has been that all messengers in the cytoplasms of eukaryotic cells (with the sole exception of the histone mRNAs) contain a length of poly(A), which fulfils some essential, although as yet unidentified function. When polysomal mRNA is examined for poly(A) content by reaction with poly(U)-sepharose or oligo(dT)-cellulose, most is bound; the fraction that is not retained has generally been thought to result from breakages in mRNA during isolation-since there is only one length of poly(A) in each mRNA, a single breakage would release a molecule altogether lacking poly(A). In two quite different systems, however, it now seems that an appreciable proportion of the cellular mRNA may lack poly(A). Working with HeLa cells, Milcarek, Price and Penman (Cell, 3, 1-10; 1974) find that about 30% of the mRNA lacks poly(A); and using early sea urchin embryos of two species (Strongylocentrotus purpuratus and Lytechinus pictus), Nemer, Graham and Dubroff (J. molec. Biol., 89; 1974) report that about 40% of the messages may lack poly(A).

To examine mRNA of HeLa cells, it is necessary both to prevent labelling of ribosomal RNA and also to exclude contaminants derived from the non-messenger RNA population. The second can be achieved by releasing mRNA from polysomes with EDTA and in these experiments the first condition was met by using fluorouridine to suppress labelling of rRNA. The labelled mRNA fraction was then passed through a column of oligo(dT)-cellulose under conditions in which more than 96% of the poly(A)-containing mRNA is retained. This divides the mRNA into a major (70%) fraction containing poly(A) and a minor (30%) fraction apparently lacking it. Both poly(A)+ and poly(A)mRNA have the same size distribution. That both classes of mRNA are in use in the HeLa cell as templates for protein synthesis is suggested by the use of puromycin, which releases both from the polysomes.

Only the demonstration that the nucleotide sequences of poly(A)<sup>+</sup> and poly(A)<sup>-</sup> mRNA fractions are different can provide satisfactory evidence that they represent two genuine cellular species and that the poly(A)<sup>-</sup> is not in fact derived from the poly(A)<sup>+</sup>. The sequences of the poly(A)<sup>+</sup> mRNA can be scrutinised by production of a complementary cDNA probe through the reverse transcription mediated by the enzyme of RNA tumour viruses; and in hybridisation

## What controls rates of evolution?

Ever since the pioneer work of G. G. Simpson some three decades ago it has been recognised that one of the most important contributions that palaeontology can make to our knowledge and understanding of evolution is in determining rates of morphological change for different fossil groups over long periods of time. Simpson was the first to bring comparative quantitative data to bear on this subject, and convincingly demonstrated that, in terms of rates of origination and extinction, and of structural innovation, the mammals easily led the field. Van Valen has now endeavoured to show, in a letter published on page 298 of this issue, that whereas in general mammals have evolved at a faster rate than other organisms, there are at least two types of evolution in which the rate is effectively the same, namely the rate of amino acid substitution in proteins and change of size measured linearly. The first type of evolution is termed the epistandard, the other the standard mode.

Van Valen attempts a tentative explanation of the epistandard mode in terms of his Red Queen hypothesis, which is spelled out fully in an earlier paper (Evolution Theory, 1, 1; 1973). Readers of Through the Looking Glass will recall that the Red Queen explained to Alice that it took all the running one can do to stay in the same place. Van Valen put forward his hypothesis in an attempt to explain why the probability of extinction of fossil groups was more or less constant in time. It is based on the idea that all species within a given adaptive zone compete intensively. A successful adaptive response by one species is assumed to occur at the expense of other species, which must either adapt by themselves speciating or become extinct, as the 'quality' of their environment is reduced. This phenomenon leads to an endless chain of adaptive responses and in the long run

means that fitness and rate of extinction remain constant.

The high rate of diversification and evolutionary turnover in mammals is likely to be the result of a variety

of factors, such as strong competitive interactions leading to specialisation in feeding methods, limitations on food supply, high mobility and energy use, interspecies aggression and territoriality. Such factors will conspire to lower the 'resource threshold' needed to prevent extinction, compared with other animals. Epistandard rates are required to make up the losses through extinction.

Though the Red Queen model might apply well to mammals, there are doubts about its more general validity. This is brought out well, for instance, in a thoughtful paper by Stanley (Systematic Zool., 22, 486; 1973), who analyses the effects of competition on evolutionary rates. Attention is confined mostly to the mammals and the bivalve molluscs (that is, clams and oysters). In sharp contrast to mammals, the bivalves are nearly all sea-bed suspension feeders which mind their own business, characterised by weak interactions with other species, primitive inflexible behaviour, uncrowded, largely sedentary mode of life and generalised feeding habits.

This has as a direct consequence substantially lower rates of evolution than mammals, as Stanley amply documents. Limits on the bivalve populations are imposed more by predation and fluctuations in the physical environment than by food resources, and biological competition is minimal. As Stanley observes laconically, "Interspecific aggression is not characteristic of bivalve behaviour". What is true of bivalves is without much doubt true of the majority of benthonic invertebrates. Through the Looking Glass might be less appropriate as a textual source for the relevant evolutionary sermon than The Walrus and the Carpenter.

A. HALLAM

experiments, the cDNA anneals back to its poly(A)<sup>+</sup> mRNA template, but does not react with the poly(A)<sup>-</sup> mRNA until much higher  $R_0t$  values are reached (and perhaps only then because of the presence of contaminating poly(A)<sup>+</sup> mRNA in the poly(A)<sup>-</sup> fraction). Because the cDNA represents only the 500 or so bases at the 3' terminal end of the messenger, immediately adjacent to the poly(A), the hybridisation analysis does not in itself exclude the possibility that the poly(A)<sup>-</sup> mRNA represents 5' ends broken off poly(A)<sup>+</sup> messengers; but this is rendered rather unlikely by the observation that poly(A)<sup>+</sup> and poly(A)<sup>-</sup> messengers are of the same size.

A similar situation is found in early sea urchin embryos, in which the sequences present in the poly(A)+ and poly(A)mRNA fractions were investigated by preparing a cDNA from the poly(A)+ mRNA by using the DNA polymerase I enzyme of Escherichia coli. At the maximum  $R_0t$  values reached, some 80% of this cDNA annealed with the poly(A)+ mRNA; the low level of reaction with the poly(A) mRNA shows that this fraction is almost entirely constituted of sequences different from those present in the poly(A)+ mRNA class. From the hybridisation reaction between the cDNA and poly(A)+ mRNA, it is possible to calculate that the complexity of the poly(A)+ mRNA corresponds to about 1,400 different species, each present on average 10,000 times in the embryo (which at this stage corresponds to about 400 cells). The complexity and abundance of the poly(A) class cannot be estimated since no probe for its sequences has been developed. Since the earlier work of Galau et al. (Cell, 2, 9-22; 1974) showed that the total polysomal mRNA falls into two abundance classes at the 600 cell stage, with less than 10% of the

mRNA representing 14,000 sequences present on average 340 times in the embryo, while most of the mRNA represents a much smaller number of sequences present in many copies each, it is of obvious importance to define the complexity of the poly(A)<sup>-</sup> mRNA and to see how the total messenger population is divided into classes containing and lacking poly(A).

Only the histone messengers previously have been shown to lack poly(A), a feature which it was thought might be related to their synthesis during only part of the cell cycle; and also which it seemed possible might be responsible for their rapid appearance in the cytoplasm after addition of a radioactive label—the delay in appearance of a label in poly(A)+ mRNA might be due to the time involved in addition of poly(A). The kinetics of production and decay of the poly(A)+ and poly(A)- mRNA classes in the HeLa cell, however, seem to be indistinguishable, so that whatever mechanism is used to produce the poly(A)" class does not promote a more rapid cytoplasmic transport. Nothing is known yet about the mechanism of production of poly(A) messages, whether a large precursor is involved as seems to be the case with poly(A)+ mRNA or whether some different mechanism is implicated. The existence of poly(A) mRNA, however, appears to argue against models which suppose that the addition of poly(A) provides the only mechanism by which messengers are recognised for transport to the cytoplasm; and also raises the question of what function the poly(A) may play in the cytoplasmic activities of the messengers containing it. The purpose of poly(A) is therefore now less clear than

BENJAMIN LEWIN

# Alpha-particle transfer

from P. E. Hodgson

Nuclear reactions in which a nucleon or group of nucleons are transferred from the incident particle to the target nucleus are one of the most fruitful sources of information about nuclear structure. Single-nucleon transfer reactions have been extensively studied and have provided detailed information about the single-particle states of nuclei. The mathematical description of such processes is much more complicated when several particles are transferred, but already much work has been done on two-nucleon transfer reactions. Reactions in which an alpha particle is transferred are also being extensively investigated, and since the alpha particle is tightly bound it is sometimes a good approximation to treat it as a single particle and to use the formalism of single-particle transfer reactions.

Nuclear reactions initiated by heavy ions often show simple features that can be quite well understood by a semiclassical theory. Thus we can think of the incident particle following a definite orbit as it is scattered by the target nucleus, as a comet is deflected by the gravitational field of the Sun. If a transfer of nucleons takes place it naturally does so when the orbit of the incident particle is nearest the surface of the target nucleus (see Fig. 1).

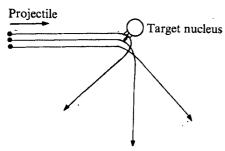


Fig. 1 Sketch showing the relations between the impact parameters, the scattering angles and the probability of a reaction taking place for several different classical orbits.

If now we consider such classical orbits of steadily decreasing impact parameter (the distance of the closest approach in the absence of a deflecting field) we can easily see that those with large impact parameters pass so far from the nucleus that a transfer reaction is very unlikely. Since these orbits are not strongly deflected, the cross section for particle transfer is small for small scattering angles. As the impact parameter is reduced the scattering angle increases and the orbit approaches nearer to the nuclear surface, increasing the likelihood of a transfer reaction. Thus the cross section of the transfer reaction initially

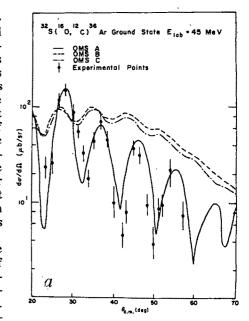
increases as the scattering angle increases. This increase continues until the orbit just touches the nuclear surface, and in this case the transfer is most likely. If the impact parameter is still further reduced the orbit cuts through the nucleus and the projectile interacts so strongly with the target nucleus that a very large number of reactions can take place and the probability of the simple transfer reaction falls. Thus on the simple classical picture we expect the transfer reaction to have a cross section that first rises to a maximum and then steadily falls as the scattering angle is

Such bell-shaped cross sections have frequently been found in studies of heavy ion transfer reactions, and semiclassical calculations give good agreement with the experimental data. These calculations are called semiclassical because the projectile is assumed to move along a classical orbit in the field of the target nucleus, but the actual transfer process is treated quantum-mechanically.

A fully quantum-mechanical theory of these reactions has also been developed, and it is known as the distorted wave theory. The interacting particles are represented by wave functions, and the way they are distorted by the field of the target nucleus is found by solving the appropriate Schrodinger wave equation. Such calculations also give the bell-shaped curve, and also some of the details of the cross sections that are not given by the classical theory (see *Nature*, 246, 334; 1973).

Last year the differential cross section of the alpha-particle transfer reaction <sup>22</sup>S(<sup>16</sup>0, <sup>12</sup>C)<sup>36</sup>Ar was measured by Braun-Munzinger and colleagues in Heidelberg (*Phys. Rev. Lett.*, 31, 1423; 1973) and they found that contrary to expectation it has a strongly oscillatory structure, as shown in Fig. 2. Calculations of the cross section of this reaction gave the usual bell-shaped curve, so it was an important challenge to try to understand the origin of the pronounced oscillations that were observed experimentally.

Charlton and Robson (Phys. Rev. Lett., 32, 946; 1974) tackled this problem and found that if it was assumed that the transferred alpha particle was in its  $2^+$  state at 28.5 MeV an oscillatory angular distribution is obtained, in good overall agreement with the experimental data. Although this process may seem somewhat unplausible physically due to the high excitation energy required, detailed nuclear structure calculations showed that the probability of the dissociation  ${}^{36}A \rightarrow {}^{32}S + \alpha^*$ , where  $\alpha^*$  stands for the excited alpha particle, is such that the transfer for the excited alpha particle



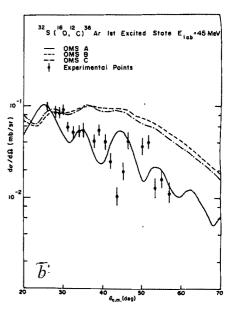


Fig. 2 Differential cross sections for the alpha-particle transfer reaction  $^{32}S(^{18}O, ^{12}C)^{38}Ar: a$ , ground state; b, first excited state, at 45 MeV compared with distorted wave calculations with the three sets of potentials in the table.

is about five times more likely than transfer in its ground state.

Additional studies of this reaction have recently been made by Werby and Tobocman (Phys. Rev., C10, 1022; 1974) and they investigated in detail the effect on the cross section of varying the potential that represents the interaction between the incident particle and the target nucleus. This is in fact not known very well, and what is usually done is to assume that it has the form of an optical model potential and to adjust its parameters to give a good fit to the differential cross section for the elastic scattering interaction, when the incident particle is simply deflected without losing energy or nucleons. Unfortunately the potentials found in this way are not

unique, and it is possible to find several sets of parameters that give equally good fits to the elastic scattering data. This is particularly so at low energies where the elastic scattering cross section is rather featureless, as is the case for the reaction of interest here.

Werby and Tobocman found several quite different potentials that all give acceptable fits to the elastic scattering, as shown in Fig. 3. The first of these labelled A has an absorbing potential of only 6 MeV, much less than the 30 MeV used by Charlton and Robson; all the parameters of these potentials are given in the table.

The cross sections for the alphatransfer reaction calculated with these potentials using the distorted wave theory are shown in Figure 2a, and it is immediately evident that the strongly absorbing potentials give curves with weak oscillations that have the overall bell shape characteristic of the classical case, while the weakly absorbing potential A gives a strongly oscillating curve that agrees very well with the experimental data. Since these calculations assumed that the transferred alpha particle is in its ground state, it is not necessary to postulate the transfer of excited alpha particles in order to obtain a good fit to the data.

These results all refer to the reaction that leaves the residual nucleus <sup>36</sup>A in its ground state. Experimental data are also available for the same reaction to the first excited state, and the corresponding measured and calculated cross sections are shown in Figure 2b. The experimental cross sections again show an oscillatory structure, though it is not so marked as for the reactions to the ground state, and once again the strongly

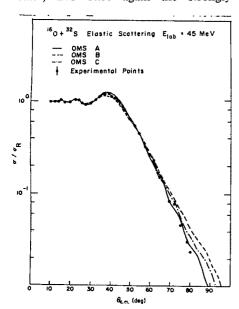


Fig. 3 Differential cross section for the elastic scattering of 45 MeV <sup>16</sup>O by <sup>32</sup>S compared with optical model calculations with the three potentials given in the table.

TABLE Optical model parameter sets.  $V = (V_0 + iw)$  $\{1 + \exp[(r - R)/a]\}^{-1}, R = r_0(A_1^{1/3} + A_2^{1/3}).$ 

Channel	Set	V <sub>0</sub> (MeV)	W (MeV)	τ <sub>0</sub> (fm)	A (fm)
16O+32S	A	40	6	1.3	0.5
<sup>12</sup> C + <sup>36</sup> Ar		40	4	1.3	0.5
16O+22S	В	40	24	1.3	0.5
<sup>12</sup> C + <sup>38</sup> Ar		40	16	1.3	0.5
15O+32S	С	100	30	1,22	0.5
<sup>12</sup> C + <sup>36</sup> Ar		100	30	1.22	0.5

absorbing potentials give a featureless bell-shaped curve while the weakly absorbing potential gives an oscillatory cross section in qualitative accord with the data.

It is interesting to try to understand just why the weakly absorbing potential gives an oscillatory cross section while the strongly absorbing potentials do not. This can be done by examining the details of the formalism of the distorted wave theory, in particular the contributions of the different partial waves to the reaction amplitude. It is found that relatively few partial waves contribute, especially when there is no angular momentum transferred in the reaction, as is the case for the present reaction to the ground state of 36Ar. Now the contribution of a particular partial wave depends on an integral of the products of the radial parts of the distorted waves of the incident and outgoing particles and a form factor depending on the positions in space of the colliding particles. The value of this integral depends on the potentials chosen, and then what determines the overall features of the cross section is the way the phase of the integral varies from one partial wave to the next for the important partial waves. It is found that for the weakly absorbing potential A the phase remains fairly constant in this region, giving constructive interference between the integrals for the different partial waves. For the strongly absorbing potentials B and C, however, the phase of the integral changes rapidly and produces destructive interference in the critical region. This difference in the behaviour of the integrals is responsible for the difference in the cross sections corresponding to the two types of potentials.

In the case of the reaction to the first excited state of <sup>36</sup>A, two units of angular momentum are transferred and this increases the number of partial waves that contribute significantly to the interaction. This has the effect of reducing the amplitude of the oscillations, but the qualitative explanation of the difference between the cross sections calculated with the two types of potentials is still valid.

It should be noted that the calculated cross sections in Fig. 2 have been normalised to the experimental data. The value of the normalisation factor depends on the product of the spectroscopic amplitudes for the dissociation

of the incident <sup>16</sup>O into <sup>12</sup>C+ the combination of α and <sup>32</sup>S t <sup>36</sup>A. If excited alpha particle also transferred the amplitudes for corresponding dissociation and bination have also to be include the calculation. At present the nucleus tructure part of the process is understood sufficiently well for relia quantitative calculations to be making and it will be a critical test of such calculations to see if they give the measured values of the cross sections.

It is clear that in spite of consider able progress there is still much to be learnt about the mechanism of these alpha-particle transfer reactions. We do not know what potential to use for the interaction between the incident particle and the target nucleus, and it is likely that further progress will come from the many attempts that are now being made to calculate the potential from a detailed theoretical model of the interaction, instead of the phenomenological approach used in the present calculations. It is clear that the elastic scattering alone does not determine the potential accurately enough, but studies of reactions such as those described here enable it to be more precisely defined. Thus it may be that studies of a wide range of reactions will also give more reliable potentials. On the nuclear structure side there is also much work to be done, and this should enable the relative contributions of unexcited and excited alpha-particle transfers to be evaluated.

The present situation for these reactions frequently occurs in nuclear reaction calculations. At first there is a strange cross-section that disagrees with existing calculations. Then a new model is developed that seems to explain the results, until it is found that several different models all do equally well. At this stage it is necessary to probe deeper and try to understand what is going on in a very detailed way, so as to distinguish between the merits of rival models.

# Movement of auxin across plant cell membranes

from our Plant Cell Physiology Correspondent

Auxins stimulate the extension fowth of plant cells. They are synthes d in the shoot apex and regulate the pansion of cells below the tip. Studies with the naturally occurring auxin indole acetic acid (IAA) and the synthetic analogue 2,4-dichlorophenoxy-acetic acid (2,4 D) have shown that these compounds move preferentially from the apex towards the base of plant stems. This process is often referred to as

'active transport' because it requires energy, and the movement of applied auxin from base to apex of stem segments is extremely low by comparison. The continued production and export of auxin from the shoot tip is thought to be responsible for such phenomena as the inhibition of outgrowth of lateral buds by the apex and the control of adventitious root production at the base of cut stems. For these reasons studies on the polar transport of auxin and the mechanism of auxin movement across the plasmalemma are of considerable interest.

Recent experiments by Rubery and Sheldrake carried out in the University of Cambridge have shown that there are at least two different mechanisms for the passage of auxin across membranes and that 'secretion' of auxin from cells may not be driven directly by the hydrolysis of ATP but rather may depend upon the pH gradient across the membrane (Planta, 118, 101; 1974).

Rubery and Sheldrake used crown gall cells of Virginia creeper grown in suspension culture as a model system to study auxin influx and efflux under a variety of experimental conditions. (Unlike most cultured plant cells crown gall cells do not require an exogenous supply of auxin in order to grow.) In a previous paper (Rubery and Sheldrake, Nature new Biol., 244,

288; 1973) it was shown that with the external medium at pH 4.3, when IAA is only slightly ionised (pK=4.7) there is a linear relationship between IAA uptake and the external concentration up to 500 µM IAA. Under these conditions it is assumed that the undissociated IAA, which is lipid soluble, enters the cells by diffusion. Once inside the cells where the pH of the cytoplasm is above pH 7, IAA would be expected to ionise, thus maintaining a concentration gradient for the passage into cells of undissociated IAA by diffusion. The authors now suggest that a passive anion carrier also operates between pH 5 and pH 6.7. The optimum for the anion carrier is about pH 6 and the carrier is apparently half saturated at quite low IAA concentrations within the range 1-5 μM. The carrier is capable of bringing about either the influx or efflux of IAA anions depending upon the direction of the concentration gradient. Studies on the operation of the carrier system were greatly aided by the observation that 2,4 D inhibits the carrier-mediated IAA anion influx and that 2,3,5-triiodobenzoic acid (TIBA), a known inhibitor of polar auxin transport, prevents the efflux of anions but has no effect upon the entry of IAA anions into cells. These results suggest that the properties of the carrier are different on the inside and outside of the plasmalemma and that 2,4 D binds to the carrier at the external face and TIBA binds at the internal face of the membrane. The apparent specificity of TIBA in inhibiting anion efflux is very interesting because it has been known for some time that in shoot segments TIBA inhibits polar auxin transport by interfering specifically with the exit of

IAA from the cells. Rubery and Sheldrake point out that if an auxin carrier similar to the one that operates in crown gall cells is localised in the membrane at the basal end of the cells in plant shoots, this would provide a mechanism for the observed polar transport of IAA. When the pH within the cell walls is acidic, auxin will enter the cells by diffusion and accumulate there. Eventually the internal anion concentration will rise above that outside the cell and the carrier will mediate the efflux of IAA anions across the membrane at the base of the cell into the cell wall. Providing the pH within the wall is lower than that of the plant cytoplasm some of the auxin will then enter the adjacent cell by diffusion where it will again be carried across the basal membrane as IAA anions. The net result of this process is polar auxin transport. Although ATP is not required to drive the secretion of IAA across the plasmalemma there is an indirect requirement for energy in order to maintain

### Density of galactic space

from David W. Hughes

THE mean density of space in the solar region of the Milky Way galactic plane is an important astrophysical quantity. First it is a fundamental parameter in the study of galactic dynamics and second it gives a lead to the way in which the galactic material is proportioned out between stars, gas and dust.

By studying the variation of galactic rotation as a function of distance from the nucleus Schmidt (Galactic Structure, University of Chicago Press, 1965) calculated that the total local mass density near the Sun should be  $0.14 M \odot \text{ pc}^{-3}$ , where  $M \odot$  is the solar mass  $(2 \times 10^{33} \text{g})$ and a parsec is 3.08×1018 cm. This is equivalent to  $1 \times 10^{-23}$ g cm<sup>-3</sup>. A similar value (~ 0.20 M ⊕ pc<sup>-3</sup>) was required by Oort (ibid.) to account for the observed stellar velocities perpendicular to the galactic plane. A problem immediately arose because the densities of stars, dust and gas in the vicinity of the Sun only added up to  $0.084 M_{\odot} \text{ pc}^{-3}$  indicating either that the estimates of the individual densities were wrong or that the Galaxy contains some mysterious mass in the form of unobserved objects.

Luyton (Mon. Not. R. astr. Soc., 139, 221; 1968) calculated how the number of stars in regions of the Milky Way vary as a function of their luminosity, and using the mass-luminosity relationship found the stellar mass density to be 0.064 M ⊙ pc<sup>-3</sup>. The interstellar dust in the Galaxy can be detected by looking at the extinction of stars as a function of distance, the reddening of starlight that passes through dusty regions, the polarisation of starlight by intervening dust and finally by observing the diffuse galactic light. The mean density of

this dust is found to be  $0.0015\,M_{\odot}$  pc<sup>-3</sup>. Interstellar gas produces atomic and molecular absorption lines in stellar spectra and also radiates emission lines in the microwave radio region of the electromagnetic spectrum. Measurements of these lead to a mean gas density of  $0.018\,M_{\odot}$  pc<sup>-3</sup>.

The missing mass problem was partially solved in 1972 by Weistrop (Astronomical Journal, 77, 849; 1972) who found that a substantial number of red dwarf stars had been overlooked previously and also that these stars are strongly concentrated towards the galactic plane. Weistrop's observations led to a 100% increase in the stellar mass density which then became  $0.13 M_{\odot}$  pc<sup>-3</sup>.

Glenn Veeder (Hale Observatories, California Institute of Technology) in a recent letter to the Astrophysical Journal (191, L57; 1974) has calculated a revised empirical relationship between the absolute visual magnitude of a star and its mass, this coming from observations of low-mass faint multiple stellar systems. Veeder finds that the main-sequence stellar mass density in the solar neighbourhood is now  $0.16 M_{\odot}$  pc<sup>-3</sup>. Add to this the mass contribution due to dust and gas and the density becomes  $0.18 M_{\odot}$  pc<sup>-3</sup> with the material divided between stars, gas and dust in the percentages 89, 10, 1.

So the missing mass of 1965 has now been found and the results of the galactic dynamicists and the stellar astronomers agree within the expected margin of error. This also means that unobserved objects (black holes, dead white dwarfs and so on) must only make up a non-significant percentage of the galactic mass.

the pH of the cytoplasm above that in the walls. Providing that this requirement is met, the specificity of the transport system would reside in the preferential distribution of IAA anion carriers in the basal membranes of the cells. It is possible that an alteration in the distribution of the carrier might also explain the lateral movement of auxin observed during tropic responses.

# Indicators of palaeoseismicity

from Peter J. Smith

DETERMINATION of the ancient geomagnetic field may be fraught with practical difficulties, but palaeomagnetists do at least have access to abundant material in which the field is recorded. Seismologists, by contrast, are less fortunate. Insofar as they are concerned with using seismic waves to investigate the Earth's internal structure and properties, they must be content with the Earth as it is today; in this sense there is no such thing as palaeoseismology. But it is possible to envisage the study of palaeoseismicity; and this branch of seismology already exists in a limited way. It is clear from plate tectonics, for example, that the bulk of the world's current seismicity coincides with the three types of plate boundary. And as the reasons for this correlation are broadly understood, it may reasonably be inferred that the association has always obtained. In principle, therefore, it should be possible to define the location and timing of palaeoseismicity by finding and dating ancient plate bound-

But this is a very general approach, equivalent in palaeomagnetism to defining when and where the Earth had a magnetic field. Is it possible to be more specific? Is there some phenomenon which could be used to determine the details of ancient seismicity or even the characteristics of individual seismic events, rather as rock magnetism may be used to determine the details of the ancient magnetic field? So far there is no known phenomenon which may be used to investigate palaeoseismicity in a routine way; but a hint that such investigations may ultimately be possible in certain situations has now come from Engelder (J. geophys. Res., 79, 4387; 1974) who has been studying the microscopic effects on surfaces which have slid against each other.

Engelder's suggestion is based on both laboratory and field studies. In the laboratory experiments, polished samples of Westerly granite were slid against each other for distances up to 3 mm and under confining pressures up to 2.9 kbar. At pressures below 0.3 kbar, stable sliding occurred with no damage to the polished surfaces. But at higher pressures stick-slip took place, producing wear grooves, scratches and small climps of gouge on the surfaces. Of particular interest are the wear grooves which are generally carrotshaped with the tip pointing in the direction of motion of the surface containing the grooves. The amount of slip can be calculated from the lengths of the grooves, for each event produces grooves with various lengths up to the distance of the slip (that is, groove lengths are not related to the total amount of slip where a series of individual slips is concerned but only to the distances of the individual slips themselves).

In the field, Engelder has found two natural counterparts to these experimental systems. The first is a slickenside (polished surface resulting from friction along a fault plane) in the Mesa Rica sandstone in the Bonita normal fault zone of New Mexico. In this case the longest of the carrotshaped grooves are 5 mm long. Unfortunately, the total slippage during the formation of this slickenside is not known, so it is impossible to say whether the grooves were produced during a single slip event or during a series of events, although the grooves do point in the known direction of motion. In the second example, a slickenside in Tintic quartzite in the Eureka fault zone of Utah, neither the sense nor the total amount of slip is known. But if Engelder is right, the sense of slip may now be deduced from the grooves. Moreover, as the longest grooves here are 0.5 mm and as 0.5 mm of slip is insufficient to generate highly polished slickensides in quartzose rock, it may be concluded that more than one slip event, and probably such events, took place.

From the morphology and lengths of those natural and experimental grooves it is possible to deduce a mechanism for their formation. In simple terms, what apparently happens is that, at the initiation of a slip, interlocking asperities (which previously prevented slip) shear. As the slip progresses, other asperities (and gouge particles) gradually plough deeper into the opposite surface, forming grooves which gradually widen (as viewed in section) and slowing down the slip until it finally stops. The cycle is then repeated, beginning again with the shearing of asperities. If the asperities did not shear at the start of each slip but instead jumped out of their old grooves and ploughed new ones, a series of inline grooves would be formed. These are seldom observed. If, on the other hand, asperities failed to shear but instead continued to plough the old

grooves, grooves would general longer than one slip length Than seldom observed either. What are served, however, are some two which are shorter than one slip corresumably because not all as cobegin to plough at the start of a

Because Engelder is here coally microscopic features, the common with palaeoseismicity is not discription tis, however, that some so is clearly related to stick-sip. Moreover, the mechanism summa above closely resembles one preceded put forward to explain macroscopic stick-slip behaviour. The question to Engelder poses is thus: is there to some preceded planes that may be used for fixing planes that may be used for fixing guishing seismic from ascismating the amount of during seismic events?

For the time being, the case of cannot be answered. But if such that as are found, Engelder's work, albeit on a microscopic scale, suggests tha trey could give a significant boost to be study of palaeoseismicity Aki () " Earthquake Res. Inst., 44, 73, 19:65 for example, has proposed a ferr la for calculating an earthquake's relative moment from the average displacement of one slip on the fault, a ferrilla which Engelder uses to determine the moment of an event along th. ... fault zone. A similar calcula i be carried out for macroscop. along large faults, although in . . . of such a scaling-up would f to be tested rigorously.

# Diseases caused by light

from a Correspondent

THERE have been advances a the 182 few years in knowledge of he 182 sensitive disease Xeroderma 182 to sum. It was inevitable the 262 to the British Photobiology So. 623 to t

The opening paper, howeve. Dr R. J. Murgatroyd (Metcoro on a Office, Bracknell) was of a ... broader nature. He discussed the varations in stratospheric ozone at a care changes in penetration of solar e -violet radiation which result from the a variations. He stressed the large rate ... variation in stratospheric czone and the lack of any consistent charse nuclear tests. Fears have recen't be expressed that a reduction in 17 12 and a consequent increase in pa ... tion of solar ultraviolet radiation and d occur with supersonic aircraft for so Dr Murgatroyd expressed the view of

# Cattle help in conservation

from Peter D. Moore

Although conservation has come to mean much more than the simple preservation of rare species, the prevention of extinction of threatened plants and animals is still an important concern of the conservationist. Apart from the possibility of aesthetic loss (consider how great a contribution the giant panda has made to the fantasy worlds of our children), a depletion of the planet's genetic resources accompanies each extinction which may ultimately be regretted even by those who have lost their aesthetic awareness. A good example of a lowly plant made rare by the destruction of suitable habitats and which has received such attention from conservationists is Dianthus armeria, the Deptford pink, which is restricted to a few meadows in southern Britain (see Walls, Proc. Bot. Soc. Br. Isl., 6. 337; 1967).

One of the sites at which this species occurs is Woodwalton Fen, a National Nature Reserve in the fenland area of East Anglia. Most of this nature reserve has been managed by man in the past, either for reed cutting, peat extraction or as grazing land; the area with Dianthus armeria was once a farmstead over which cattle were grazed. This practice was not continued in the early days of the reserve's history, but locally the pressure of rabbit grazing was sufficient to maintain the shortgrass community in which Dianthus survived. Following the myxomatosis epidemic of 1954, however, extensive changes occurred in the vegetation of the area; coarse grasses, particularly Calamagrostis epigejos and Agropyron repens, spread at the expense of shorter-growing species, including Dianthus. By 1964 only five plants remained and at this stage a new management regime involving grazing by Friesian heifers was introduced. Subsequently the population of *Dianthus* expanded gradually to 900 plants by 1967.

Williams, Wells and Wells (J. appl. Ecol., 11, 499; 1974) have now attempted to analyse the respective roles of cattle and rabbit grazing in this particular recovery. They undertook a quantitative analysis of the faeces of these animals by concentrating the surviving plant epidermal fragments in a faecal sample and spreading them on a microscope slide. The species composition of these samples was subsequently estimated by means of randomly located point observations. This type of analysis was repeated periodically through the course of a year in order to determine the changing food preferences of the animals during that time. As Stewart (J. appl. Ecol., 4, 83; 1967) has pointed out, there are many problems associated with this technique, particularly the relative digestibility of different species. But the preponderence of coarse grasses in the cattle faeces and the virtual absence of dicotyledonous species gives a reasonable representation of this animal's diet. The rabbits also seem to have neglected dicots, but show a stronger preference for the less coarse grasses, such as Festuca.

The results of these studies suggest that the beneficial effect of cattle upon the Dianthus armeria population results from their selective grazing upon the more aggressive species of grass. The techniques employed however, do not permit conclusions regarding the influence of trampling upon Dianthus by increasing microtopographical diversity. When scarce, the plant became restricted to ant hills, which suggests that soil disturbance and microtopographical factors may be important in controlling its distribution. Examination of the germination and establishment requirements of Dianthus under laboratory conditions might assist in the separation of these two distinct influences of cattle grazing. Meanwhile it cannot be denied that this is an efficient management practice for the conservation of a diminishing species.

discussion that these fears have been exaggerated.

Recent work on the genetic basis of Xeroderma Pigmentosum have shown the value of studies of heterokaryons formed by fusing fibroblasts from patients with the disease and from normal subjects. Dr P. Lohman (Netherlands Organisation for Applied Scientific Research, Rijswijk) reported that there are now five complementation groups in Xeroderma Pigmentosum. Dr Lohman also discussed experi-

ments which showed that ultraviolet-irradiated chicken fibroblasts can repair DNA damage other than that involving pyrimidine dimers. Investigations by Dr F. Giannelli (Guy's Hospital Medical School, London) indicate that the enzyme which is defective in most patients with Xero-derma Pigmentosum is probably not a monomer. In addition, it is possible to detect heterozygotes for Xeroderma Pigmentosum mutations by the assessment of DNA repair in heterokaryons.

The past few years have seen the emergence of Xeroderma Pigmentosum variants, patients with clinical features of the disease but with normal ability to carry out excision repair of DNA. Dr A. Lehmann (University of Sussex) has recently confirmed that in these patients all of the steps in excision repair are normal, but that there is a defect in post-replication repair. Post-replication repair in Xeroderma Pigmentosum variants is inhibited by caffeine, an effect which is not present in normal cells. Cells from patients with Xeroderma Pigmentosum who are defective in excision repair show changes in post-replication repair which are intermediate between normal and Xeroderma Pigmentosum variants.

Another aspect of this disease was discussed by Dr C. A. Ramsay (Institute of Dermatology, London). He demonstrated the abnormal reactions of the skin of these patients when they are irradiated with monochromatic ultraviolet light in the 290-320 nm region. The delay in the onset of erythema after irradiation may enable the diagnosis of Xeroderma Pigmentosum to be suspected early in life. In addition, Dr Ramsey reported the first successful case of the prenatal diagnosis of Xeroderma Pigmentosum by the detection of the deficiency of excision repair in cultured amniotic cells. Xeroderma Pigmentosum is the only condition to date in which excision repair of DNA has been found to be abnormal. Actinic keratoses occur on normal skin as a result of chronic exposure to solar ultraviolet irradiation and Dr J. Pitts (University of Glasgow) has found that in cells of such keratoses excision repair is not impaired.

Several other aspects of photosensitive skin disease were discussed. A review of the value of action spectroscopy in dermatology was given by Dr Frain-Bell (University of Dundee). The photodermatoses present many unsolved problems not least of which is that of treatment. Dr J. C. van der Leun (State University of Utrecht, Netherlands) has used the technique of repeated irradiation of the skin as a method of treatment and has found it of benefit in solar urticaria and polymorphic light eruption. These findings are of interest because the two diseases represent quite different reactions to sunlight; solar urticaria occurs very soon after irradiation whereas in polymorphic light eruption there is a delay of several hours before the skin changes

Finally, a detailed study of the photochemical basis of a dye-induced photodermatitis was reported by Dr K. Davies (University of Salford). He showed that in this reaction with an antraquinone dye, singlet oxygen is an important intermediate.

### review articles

# Do machines understand more than they did?

Yorick Wilks\*

To be able to communicate in ordinary English, a machine must have access to common sen information about the real world. The first computer systems to attempt such communication lived very limited worlds. Now, with a "second generation" of programs, those worlds are widening although the exact significance of the recent advances is still in dispute.

In his report to the Science Research Council on the state of artificial intelligence (AI), Sir James Lighthill¹ gave most of the field a rather bad prognosis. One of the few hopeful signs he saw was Winograd's computer system for natural language understanding². Yet now, only a year later, Winograd has stopped work on the system he constructed, and has begun a new one on entirely different principles. He went so far, in a survey lecture (Third International Joint Conference on Artificial Intelligence, Stanford, California, 1973) of extraordinary modesty in a field not known for its small claims, to place his celebrated early work in only the "first generation" of computer systems designed to understand natural language, before going on to describe others' "second generation" systems.

I shall return later to this metaphor of generations, but what is one to say in general terms of a field where yesterday's brightest spots are today's first generation systems, even though they have not been criticised in print, nor shown in any generally acceptable way to be fundamentally wrong? Part of the answer lies in the profound role of fashion in artificial intelligence in its present prescientific phase. A cynical American professor remarked recently that AI had an affair with someone's work every year or two, and that, just as there were no reasons for the falling in love, so, later, there were no reasons for falling out again. In the case of Winograd's work it is important now to resist this fashion, and re-emphasise what a good piece of research it was, as I shall show in a moment.

Another part of the answer lies in the still fundamental role of metaphysical criticism in AI. In the field of computer vision things are bad enough, in that anybody who can see feels entitled to criticise a system, on the ground that he is sure that his own vision is not based on the same principles. In the field of natural language understanding things are worse: not only does anyone who can speak and write feel free to criticise on the corresponding grounds, but those trained in disciplines parasitic upon natural language, linguists and logicians, often have strong views on a priori grounds on how things must be done. It is this metaphysical aspect of the subject that gives its disputes their characteristically acrimonious flavour.

In this article I want to sort out a little what is agreed and what is not; what are the outstanding disputes and what are the systems making the most testable claims. If what follows seems unduly philosophical, it should be remembered that little is agreed, and almost no achievements are beyond question. To pretend otherwise, by concentrating only on the details of established programs, would be meretricious and misleading.

#### Winograd's understanding system

Winograd's system runs as a dialogue, in real time, between a human operator who types in messages, and the system proper,

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called SHRDLU, which displays on a screen pictures of a closed world of coloured blocks and pyramids, a box into which they can be put, and an arm or crane that can move the objects about. SHRDLU displays its replies to the operator by writing on the same screen. The following is a discontinuous sample of the dialogue Winograd gives in his book. SHRDLU's replies are in upper case:

pick up the big red block

OK

find a block which is taller than the one you are holding and put it into the box.

BY 'IT', I ASSUME YOU MEAN THE BLOCK WHICH IS TALLER THAN THE ONE I AM HOLDING

OK

what does the box contain

THE BLUE PYRAMID AND THE BLUE BLOCK

had you touched any pyramid before you put the green one on the little cube

YES, THE GREEN ONE

when did you pick it up

WHILE I WAS STACKING UP THE RED CUBE, A LARGE RED BLOCK AND A LARGE GREEN CUBE

why

TO GET RID OF IT.

This ingenious program was also able to accept new definitions of words, such as "steeple"; to make use of what it knew about the properties of the objects described, such as that a block could have its top cleared off, but a pyramid could not; and also to remember what it had done before, as in the sample above.

The program was written in the language PLANNER<sup>3</sup>, which is the concrete expression of the slogan "meanings are procedures", a sentiment into whose own meaning it is probably best not to enquire too closely, but which has undoubtedly led to a new style of programming. PLANNER is a theorem-proving language: it tries to establish the truth of assertions, not in the normal uniform, proof-theoretic, manner, but by accepting a range of "programmed hints" about how best to proceed at any point. In a language understanding program like Winograd's this means replacing familiar grammar rules such as:

 $S \rightarrow NP + VP$ 

(a sentence consists of a noun phrase followed by a verb phrase) by procedures, in this case:

((PDEFINE SENTENCE ((PARSE NP) NIL FAIL)((PARSE VP) FAIL FAIL RETURN)))

The details of the notation need not detain us; what is important is that Winograd's grammar is not the conventional list of rules, but small sub-programs like the line above, that actually represent procedures for imposing the desired grammatical structure. The definitions of more complex words are also in this form: here, for example, is the "theorem" defining the meaning of "pickup".

(DEFTHEOREM TC-PICKUP(THCONSE (X (WHY (EV))EV)

(#PICKUP \$?X)(MEMORY)(THGOAL(#GRASP \$?X) (THUSE TC-GRASP))

(THGOAL (#RAISEHAND)(THNODB)(THUSE TC-RAISEHAND))

(MEMOREND (#PICKUP \$?EV \$?X))))

Once again detailed explanation of the notation is not needed to make it clear that the word is being defined in terms of a number of more primitive sub-actions, such as RAISEHAND, each of which must be carried out in order that something may indeed be picked up. The linguistic content is a "systemic grammar"<sup>4</sup>; plus a simple system of semantic "features" marking words and arranged hierarchically, such as PHYSOB (for physical object words) and ANIMATE (for animate words like "robot"); together with some factual knowledge about the block world. Both types of knowledge, linguistic and factual, are represented in PLANNER and the program has access to whichever sort is required at any given moment, rather than proceeding in the conventional manner by first parsing to get a syntactic structure and then manipulating the features to get a semantic structure.

#### Significance and shortcomings of SHRDLU

One reason for the enormous impact of this work was that, before its appearance, AI work was linguistically trivial, while • the systems of the linguists had no place for the use of inference and real world knowledge. Thus a very limited union between the two techniques was able to breed considerable results. Before Winograd there were few programs in AI that could take a reasonably complex English sentence and ascribe any structure whatever to it. In early classics of "natural language understanding" in AI, such as Bobrow's STUDENT<sup>5</sup> problem solver for simple algebra, input sentences had to be short and of stereotyped form, such as "what is the sum of . . . ?" Conversely, in linguistics, there was, until very recently, little speculation on how we understand the reference of pronouns in such elementary sentences as "The soldiers fired at the women and I saw several fall," where it is clear both that the answer is definite, and that finding it requires some inferential manipulation of generalisations about the world. The reader should ask himself at this point how he knows the referent of the pronoun in that sentence.

So far, the reaction to Winograd's work has been wholly uncritical. What would critics find to attack if they were so minded? First, that his linguistic system is highly conservative, and that the distinction between "syntax" and "semantics" may not be necessary at all. Second, that his semantics is tied to the simple referential world of the blocks in a way that would make it inextensible to any general, real world, situation in which, for example, "block" might mean "an obstruction" and "a mental inhibition", as well as "a cubic object". The ambiguity of "in" in "He ran the mile in five minutes", and "He ran the mile in a paper bag", would also seem to be beyond its powers of resolution.

Third, the blocks world is strongly deductive and logically closed. If gravity were introduced into it, then anything that was pushed in a certain way would logically have to fall. But the common sense world, of ordinary language, is not like that: in the "women and soldiers" example given earlier, the pronoun "several" can be said to be resolved using some generalisation such as "things shot at and hurt tend to fall". There are no logical imperatives there, even though the meaning of the pronoun is perfectly definite. One might summarise these criticisms by paraphrasing a central argument of Wittgenstein's that the interesting point is not the creation of a language of the blocks world sort, but how different it is from our own.

Indeed, it might be argued that Winograd's system is not about natural language at all, but about how goals and subgoals are to be organised in a problem-solving system capable of manipulating simple physical objects. The definition of "pickup" quoted above is in fact an expression of a procedure for picking up an object in the SHRDLU system. Nothing about it would help one understand the perfectly ordinary sentence "I picked up my bags from the platform and ran for the train."

On the other hand, it has not been shown that the language

facilities just outlined cannot be incorporated in the linguistic structures that SHRDLU manipulates; and even if they could not, the work still would be significant by virtue of its original control structure and its demonstration that real world knowledge can be merged with linguistic knowledge in a working whole.

#### Paradigms and procedures

Winograd's work is a central example of the "artificial intelligence paradigm of language", using "paradigm" in Kuhn's sense of a large scale revision in scientific thinking, where the paradigm revised is the generative paradigm of the Chomskyan linguists8. From the AI point of view, the generative linguistic work of the last fifteen years has three principal defects. First, the generation of sentences, with whatever attached structures, is not in any interesting sense a demonstration of human understanding, nor is the separation of the wellformed from the ill-formed by such methods; for understanding requires, at the very least, both the generation of sentences as parts of coherent discourse, and some attempt to interpret, rather than merely reject, what seem to be ill-formed utterances. Second, Chomsky distinguishes between performance models, on the one hand, and competence models which are the province of serious linguistic study, on the other. Whatever his intention, this had had the effect of isolating modern generative linguistics from any possible test of the systems of rules it proposes, since testing necessarily involves performance. AI, though it too is concerned with the structure of linguistic processes per se, is equally interested in their implementation. Third, there was until recently no place in the generative paradigm for inferences from facts and inductive generalisations, even though very simple examples demonstrate the need for it.

These points would be generally conceded by those who believe there is an AI paradigm of language understanding, but there would be far less agreement over the positive content of the paradigm. The trouble begins with the definition of "understanding" as applied to a computer. At one extreme are those who say the word can only refer to the performance of a machine: to its ability say, to sustain some form of dialogue long enough and sensibly enough for a human interrogator to be unsure whether what he is conversing with is a machine or not. The majority, however, probably argue that this is not enough: the methods and representations of knowledge by which the performance is achieved must be of the right formal sort, and mere performance based on ad hoc methods does not demonstrate understanding.

This issue is closely related to that of the role of deduction in understanding natural language, simply because deduction is often what is meant by "right methods". The dispute over deductive methods on the one hand, and other inferential systems closer to common sense reasoning on the other, is in many ways a pseudo-issue because it is so difficult to define clearly what a non-deductive system is: almost any set of formal procedures can be expressed deductively. The heart of the matter concerns the most appropriate form of representation of knowledge, rather than how that knowledge could conceivably be expressed, and it may well turn out that the most appropriate form is nondeductive. The same insight has largely defused another heated issue; whether the appropriate representations should be procedures, as in Winograd's work, or declarations. One disadvantage of procedural representations is that if each item of "knowledge" is attached to a procedure for achieving a particular goal, it will have to be stored separately for each different goal for which it may be used; and if you want to change it later, it will have to be changed separately for each. Another is that conventional, declarative grammars are easier to understand; and Winograd's procedural grammar has been rewritten in more conventional form9 for that reason.

While there is now general agreement that any system should show how it is actually to be applied to language, it is no longer fashionable to demand that it must be written in a procedural language, like PLANNER.

#### "Second generation systems"

When Winograd contrasted his own with what he called second generation systems, he meant, as always in this subject, generations of fashion, not chronology or inheritance of ideas. The foundations and terminology of the approaches he discusses were set out in print in 1966, 1967 and 1968 (refs 10–12). In an already very influential recent paper (unpublished but available from the Artificial Intelligence Laboratory, MIT), Minsky has drawn together strands in the work of these authors and of Charniak<sup>13</sup> using a terminology of "frames":

"A frame is a data-structure for representing a stereotype situation, like a certain kind of living room, or going to a children's birthday party. Attached to each frame are several kinds of information. Some of this is information about how to use the frame. Some is about what one can expect to happen next. Some is about what to do if these expectations are not confirmed."

"We can think of a frame as a network of nodes and relations. The top levels of a frame are fixed and represent things that are always true about the supposed situation. The lower levels have many terminals—"slots" that must be filled by specific instances or data. Each terminal can specify conditions its assignments must meet . . . Simple conditions are specified by markers that might require a terminal assignment to be a person, an object of sufficient value etc . . ."

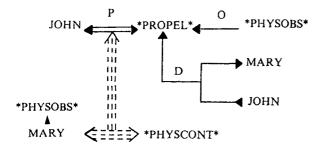
The key point about such structures is that they attempt to specify in advance what is going to be said, and how the world encountered is going to be structured.

In psychological and visual terms, frame approaches envisage an understander as at least as much a looker as a seer. The new work which owes most to Minsky's advocacy is Charniak's. He studied what sorts of inferential information would be needed to resolve pronoun ambiguities in children's stories, and in that sense to understand them. One of his example "stories" is: "Jack was invited to Jane's birthday party. She wondered if he would like a kite. A friend told Jane that Jack already had a kite, and that he would make her take it back".

The problem is to decide whether the penultimate word "it" refers to the first kite mentioned or the second. Charniak's system does not actually run as a program, but was a theoretical structure of rules called "demons" that correspond roughly to what Minsky later called frames. A demon for this example would be "If we see that a person might not like a present X, then look for X being returned to the store where it was bought".

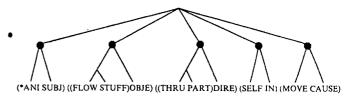
The other systems within the frames approach are rather different. Instead of resting on representations that are superficial in the sense, in which the demon above is just a sentence in English (or in some item-to-tem correspondence to one), they are based on reduction to a system of linguistic primitives, and it is at that level that the understander's computations must take place.

Schank's system takes English sentences and produces paraphrase style inferences from them. So, from "John strangled Mary", it produces "John caused Mary to die", "John placed his fingers about Mary's throat" and so on. His internal structures, that he calls conceptual dependencies, may be exemplified by the following for "John hit Mary".



The items between asterisks are primitive actions, a different types of arrow denote different types of relation

My own system, which I call preference semantics, runs an English-French machine translation system, which proa very clear test of the correctness of the inferences. example, from "The monkeys ate the bananas although were not ripe, because they were very hungry" it would tran the two "they's" correctly by different French pronouns beca of what it knows about hunger and ripeness. It does this looking for the most coherent representation in which conce find their "preferred connections" to others. In this ca "ripeness" prefers to be of plants though the system would n balk at "The old man was ripe with years" where that preferencannot be satisfied. It will also tackle examples like the "wome and soldiers" one, where it has to bring to bear rules of partia inference about the usual course of the world. These inference rules pattern-match and chain together complex structures of primitives called templates: networks whose nodes have tree structure like the following one for the action "drink":



#### (\*ANI SUBJ)((FLOW STUFF)OBJE)((THRU PART) DIRE)(SELF IN)(MOVE CAUSE)

This is to be interpreted, from the left, as drinking is a causing to be of a preferred liquid object (FLOW STUFF), by a preferred agent that is animate \*ANI, and of causing the liquid to be inside the animate thing (SELF IN), via an aperture (THRU PART). The important difference between this system and Schank's on the one hand, and Winograd's on the other, lies not in the semantic markers used, but in the ways they are related in advance in the semantic structures. So, for example, the understander could infer from the above tree structure for "drink", when incorporated in an appropriate template, that, after the act of drinking, the liquid (whatever it is) is inside the drinker. Such an inference would be essential to the resolution of the pronoun in a sentence like "John drank no gin in his cocktail but it felt warm in his stomach nonetheless".

#### Outstanding points of contention

There are many points of dispute between the proponents of second generation systems: such as whether one should make massive forward inferences as one goes through a text, keeping all one's expectations intact, as Charniak and Schank hold, or whether, as I hold, one should adopt some "laziness hypothesis" about understanding, and generate deeper inferences only when the system is unable to solve, say, a referential problem by more superficial methods.

But the most important argument concerns the question of level: whether the representation should be superficial and highly particular, or deeper and more general. Schank and I hold that the the core of understanding consists of very general, and sometimes almost vacuous, inferences about human behaviour, desires and expectations, manipulation of which requires a highly structured primitive representation, and not just the English-words-plus-variables approach of Charniak. This becomes very clear when one actually builds a system that takes English input and deals with it from scratch. Charniak and Minsky believe that this initial "parsing" can be effectively decoupled from the interesting inferential work and simply assumed. But that is not so, because the initial parsing actually depends on many of the later inferences, so the assumption of decoupling can lead to something like a circularity. For example, in analysing "He shot her with a colt", we cannot ascribe any

structure at all until we can make the inference that guns rather than horses are instruments for shooting.

Another distinction arises on the question of syntax analysis. Syntax, which is the heart of Winograd's system, is discounted by both sorts of frame approach, though for very different reasons. Charniak discards it because it is part of the initial parsing from which his inferential work has been "decoupled"; Schank and I do so because we believe semantic analysis to be fundamental, and that in an actual implementation the results of syntactic analysis can all be achieved by a sufficiently powerful semantic analyser. And this last assumption is confirmed by the limited degree of success that the two semantic analysers have actually achieved.

Closely connected with the dispute over the appropriate level of representation of knowledge is another as to what is the most pressing task in the immediate future. I would maintain strongly that work on understanding systems needs a well defined task somewhere between the trivial and the fantastic. If first generation systems sometimes seem trivial, then some of the goals of the more recent work seem fantastic, and at the present stage of our ignorance I would include in that category analysis of any prose, whether children's stories or whatever, that appears to contain problems for the average reader. I find I have to think. about the right analysis of Charniak's kitestory, discussed above. This is not true for most sentences, even though it is genuinely difficult to specify uniform rules for their analysis. I consider concentration on problem examples, to the exclusion of more natural language, to be a bad inheritance from AI's problemsolving past. On the other hand, not all understanding can be tackled by deep semantic representations: any machine understander that analysed the sense of "good morning" in order to find the correct reply would be making a fundamental error.

Again, a reasonably fluent speaker of, say, German, might well not understand a German conversation about birthday presents without detailed factual information about how Germans organise the giving of presents. Conversely, an expert might understand much of a technical article even with very little

knowledge of the language in which it was written. These considerations tell for Charniak's approach, and it is perhaps paradoxical that the sort of natural language understander that would tend to confirm his assumptions would be one concerned with discourse about, say, the details of repairing a motor car, where factual information is what is central, yet he has concentrated on something as general as children's stories, which need deeper assumptions about human desires and behaviour. That has sometimes seemed to move his research towards the fantastic, and has so far prevented its actual implementation.

In the end the differences about level of representation may again turn out to be ones of emphasis and of what is most appropriate to different subject areas. We should soon see whether that is the case, or whether one "frames approach" is right and the other wrong. It would be nice if this were to be settled by computation rather than by another change of fashion.

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# Where is experimental research on earth strain?

P. H. Svdenham\*

Progress in experimental earth-strain research depends crucially on the measurement capability of its instruments, which are here subjected to critical review.

SERIOUS high sensitivity measurement of earth strain began forty years ago and many interesting experiments have since been proposed. A large number of research themes still await study, however, because of the lack of suitable instruments and installation experience. This article provides an overview of the experimental aspects, including rival mechanical and optical designs, in order to compare their shortcomings and provide us with guides for future research.

What is needed is a measurement capability like that provided by magnetometers or inertial seismometers which can measure a large number of parameters within hours of arrival at virtually any site.

#### Information to be desired

Depth of penetration of a radiation wave into the Earth increases with signal period. Gravitational potential is, therefore, valuable in investigations of the Earth's deep structure because it provides well isolated forcing frequencies ranging in period from hours to years. It is now virtually routine to extract the frequency spectrum from the original

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time domain strain record and numerical examination of this can yield data about the composition of the crust and mantle, and about earth resonances.

Complete specification of regional strain induced in rock requires knowledge of six strain components. If the directions of the principal axes of strain are known the monitoring task can be simplified because fewer components need measuring. At present, neither applied theory or instrument siting procedures are sufficiently advanced for making accurate assumptions about the axes. Indeed, recent reports, concerned with unexpected results obtained with tidal tiltmeters1,2 and strainmeters3, indicate that an instrument sees a regional component that can be much modified by the local structure and measurement cavity shape.

If experimental measurements lead us to generalised rules about the direction of the principal axes (criteria which hopefully need no measurements at each site) this should make it possible to investigate regional effects with less than six strainmeter components. Until more is known about this problem it hard to see how isolated strainmeters (singly, in colinear arrays or otherwise) can be used to study regional phenomena.

Theoretical studies will certainly help but multi-com-

ponent tensor arrays of strainmeters must be used to establish the components at each point: large area strain should not be extrapolated for data from a single strainmeter.

A closely related question is that of strainmeter length. No evidence so far presented proves which interval is optimum. Earlier reports favoured long intervals to average out varying rock properties, but comparative records4 now seem to show that length is less critical than was thought. If metre-length gauges could be used it would considerably ease site selection. The first reports of satisfactory short mechanical instruments are published in this volume4,5. Although the feasibility of surface installations<sup>6,7</sup> has been clearly demonstrated with laser-based instruments the sophistications used contrast sharply with a simple mechanical instrument installed in an underground chamber. It is doubtful if theory alone will be able to provide a close enough model of real local-rock situations for strains are induced by many varied processes. Tensor arrays of cheap instruments could well provide the sought-after generalisations for considerably less effort.

Allied questions are: does regional (fractured, inhomogeneous in practice) rock deform in a truly elastic manner? Are there significant mechanical hysteretic effects? How does fractured hard rock respond? King<sup>8</sup> has suggested that soft rock locations are preferable but this has not been verified in practice.

Few positive statements can be made about such problems: some answers are beginning to emerge. Mounting methods, for example, show that the technique used to couple the instrument to the rock is of little importance once an adequate settling time has passed. An instrument can now be installed within the hour to provide useable strain data within days, which represents a considerable improvement over methods of a decade ago.

Before regional effects can be confidently investigated experience must be gained in setting up an instrument consistently. As earth strains do not repeat with time it is of little value to reinstall an instrument to check validity. Instead the simultaneous comparison of two or more instruments on a common Earth interval or a special purpose test base must suffice. Until quite recently this powerful metrological procedure was totally ignored.

Reports of comparisons between instruments are rare. Is this because few attempts have been made or because few have been successful? The seven day runs of the first Invar-catenary instrument and a laser interferometer compared well<sup>10</sup> for most (but not all) of the period. Vali *et al.* published<sup>11</sup> the result of a 36-h run obtained with an interferometer and a quartz-tube instrument, but they contain major discrepancies. It is only in the last year that reasonable multiple recordings on a common rock interval have been reported<sup>4,16</sup>. In both instances the duration of records was long enough to provide confidence in routine use of strainmeters.

Dipping plates at continental boundaries should be subject to abnormal strains. Strainmeters for studying this would need to be very stable and reliable. Any measurement proposals for testing the theory of an expanding Earth<sup>13,14</sup> fall in this category.

The rate of dissipation of tidal energy has been calculated at about 10<sup>19</sup> erg s<sup>-1</sup>. As the resonance quality factor of the solid earth is several orders of magnitude larger than for the oceans, only a small percentage of this energy is dissipated in the solid-tide vibrations. Study of solid tidal signal phase variations might yield an appreciation of the magnitude and uniformity of the energy loss process. Indirect progress toward solid tidal measurements has been made<sup>15</sup> through a better understanding of the ocean loading effects that may mask the expected small phase delays.

There are many other problems. How deep do surface thermal strains really propagate? How important is the adiabatic heating (induced by ambient pressure changes) on the instrument and the measured rock interval? How local topography related to the directions of the prix strain axes? How far can the individual strainmeters tensor array be separated if a regionally represent tensor is to be obtained? .

#### Are strainmeters good enough yet?

To look at the current state of nanostrain measureme it is necessary to study the fundamentals of each designoring the sophistications that can veil the principl Strainmeters consist of a length standard that is comparagainst a rock interval using a micro-displacement detetion method. The standard used in geophysical strainmeter may be mechanical or optical.

The first mechanical nanostrain gauge used a steel pipe, 20 m long, as the standard with an electromechanical velocity detector. This work by Benioff, in 1935, provided the first tidal records to justify theoretical predictions of rock tide. By 1950 the relatively poor thermal coefficient material—steel—has been replaced by 50 mm diameter fused-silica tube. Many similar instruments have been commissioned since then<sup>18</sup>. The choice of transducer is a matter of personal preference for inductive, capacitance and optical alternatives can each provide the sub-nanometre resolution needed<sup>17</sup>.

In 1951 Sassa reported an alternative method using an Invar wire suspended between two pillars<sup>18</sup>. A mass hung in the centre moves ten times as much vertically as it does horizontally. There are, however, problems of gain variation with sag change. Today there is little need to incorporate mechanical amplification as electrical transducers are, able to provide adequate gain<sup>17</sup>. A still simpler wire method<sup>19</sup> consists of an Invar wire hanging in an horizontal catenary. A beam balance provides the necessary tension and monitoring facility. Rotation of the balance, as the rock interval varies, drives the transducer.

This method rapidly gained acceptance<sup>20,22</sup> and about seventy instruments are now in use as the cheapest and easiest strainmeter design available. The Cambridge group operate the majority of operational catenary instruments<sup>16</sup>, having installed them in many different global locations.

The chief difficulty in the original catenary design was the relatively large creep in length of the Invar<sup>21,23</sup> for this required frequent readjustment to maintain the record on-scale. Automatic chart ranging was constructed by two groups<sup>20,24</sup> to overcome this. (Drift from this cause reduces the precision of secular earth drift estimates and other long-period phenomena.) In many ways<sup>22</sup> Invar is not the best material for this purpose. An improved form of tensioned-standard meter has been developed with fused-silica canes linked together as a chain<sup>12,25</sup>. It was found to exhibit considerably less creep than Invar, improved dynamic performance and better tolerance to thermal contamination. It is important to note that no mechanical strainmeter has been provided with environmental control measures always used in laser instruments.

An interferometer can be used to detect small movements between a distant reflector and a beam splitter or reference reflector of Michelson and Fabry-Perot forms. Claims that this is the almost perfect strainmeter cannot be accepted. Whichever design is used three requirements must be met. First, the radiation must be stable in wavelength to the same order as the drift required (10<sup>-10</sup> h<sup>-1</sup> at least). This calls for the use of the new molecular absorption stabilised lasers. One of the four operational laser designs uses absorption in methane<sup>26,27</sup>, another in iodine<sup>28</sup>; one uses a mechanical etalon cavity<sup>6,7</sup>, a second iodine stabilised unit will be operational shortly<sup>28</sup>. On this factor alone it is hard to envisage cost reductions reaching a point comparable with mechanical designs. Reliability is also a parameter to be reckoned with at present.

The second necessity is to stabilise the radiation path

because ambient pressure, humidity and temperature variations alter the refractive index (and hence apparent strain) to a degree similar to the effects of environment on a fused-silica standard. An adequate environment is generally provided for optical meters by evacuating the optical path an expensive investment that is costly to maintain.

The third requirement is to use the interferometer principle for making a transverse comparison with the mechanical rock interval. This involves the use of mechanical components-mounts, optical elements and holders for same. As these are within the measurement loop they may introduce creep and environmental errors which are not easily quantified. One study of Fabry-Perot etalon stability indicated30 that the reflecting film changes were producing cavity length changes of 10<sup>-8</sup> per month.

Other claims must be carefully considered—that of wide bandwidth, for instance. Counters can respond to 10 MHz with ease but fringe-counting alone rarely provides adequate resolution (1 km interval just suffices). Opto-mechanico interpolation can divide the fringe to 1% but introduces bandwidth limiting mechanisms. The problem is overcome by using methods that provide a beat frequency output but, even so, the frequency stabilisation servos involve the use of bandwidth limiting mirror drivers. Further, the mechanical mounting elements possess natural resonances around 1 kHz so it is unrealistic to talk of extremely wide bandwidth. Furthermore, wide bandwidth is a hollow criteria for crustal movement research rarely needs better than one second response time.

With so few laser instruments running7,27,28 it is perhaps still too early for an assessment, but they seem to be inferior to mechanical devices. Several reports include statements about secular strain change: 10<sup>-10</sup> per day<sup>26</sup> and 10<sup>-9</sup> ground compression<sup>6</sup>. These statements are of limited value for observed drift rates cannot be attributed to ground or instrument as separate entities. (Similar overall drift performance has been achieved12 with fused-silica instruments.)

#### Ultimate stability—mechanical or optical?

A mechanical length standard is generally regarded as inferior to an optical one. Recent research, however, has shown that fused-silica can achieve a similar drift rate as the laser method showing that both need consideration in earth strain applications. Brownian movement of the internal molecules of a mechanical object provides a theoretical limit to its length stability. Relative size changes due to this would be around 10<sup>-15</sup> (one second averaging time) and experimentalists have reached this limit in small devices. It is yet to be shown that a relatively long mechanical bar can be made as stable. The relative wavelength instability of laser radiation can be ascertained from theoretical considerations; the limit is around 10-14 for a 1-s integration time. As with mechanical standards this has yet to be reached; practice is currently limited to  $10^{-12}$ instability.

It seems, therefore, that there is little to choose between the two for stability, experimental art being the deciding factor at present.

#### The ultimate strainmeter?

Mechanical instruments are cheap but prone to environmental effects. A moderate increase in cost could provide the controlled surround which all laser devices have. Experience gained25 with test-base temperature control suggests that microKelvin stability is achievable and that the cost and reliability would be comparable with the vacuum system of a laser strainmeter. Passive31, rather than active water circulation, control would further reduce the cost.

Another course of action could be to use ultra-low expansion materials for the standard (3 10-8 K-1 thermal expansion coefficient).

A third, already used, alternative is to monitor the

influencing parameters, correcting the data or physical standard accordingly. This method does not protect the standard from stress cycling—a condition that introduces mechanical hysteresis strain changes. Furthermore it is hard to ensure that the correct parameter is monitored.

These above approaches seem logical but, in fact, do not meet the basic needs of the measurement problem. The current unstated aim is to devise a zero temperaturecoefficient instrument placing it in existing underground apertures. On two counts this is incorrect procedure.

First, as the chamber must be relatively large it will modify the parameter sought. Second, long term thermal changes to the chamber will also alter the rock dimensions introducing contaminating components in the record. Ultimately it will become necessary to correct for this.

It seems more logical to opt for an instrument that is submerged in the rock in an insignificant size aperture and which has its overall temperature-coefficient matched to the host rock. It has recently been shown that 1 m fusedsilica and Invar strainmeters are feasible so these could be installed into deeply drilled core holes arranged as a shell-burst to provide six components at one place. As the length-standard would always be at the same temperature as the rock, the thermal noise should be reduced without recourse to control of its environment.

Only when it is firmly established what strainmeters measure and when we are able to place them to measure what we need will we be able to significantly advance our knowledge of the deeper Earth. Earth strain measurements are, at last, beginning to be meaningful now that the basic requirements of the problem have been recognised.

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# articles

# Deep drilling in an active geothermal area in the Azores

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A deep borehole on São Miguel encountered temperatures exceeding 200° C at a depth of 550 m. Subaerial volcanics persist to a depth of 786 m below sea level and indicate an average subsidence of 0.1 cm yr<sup>-1</sup> for the island over the past 690,000 Myr.

DEEP drilling is essential to the understanding of the process of the formation of oceanic islands. The petrology and geochemistry¹ of island rocks differs significantly from those of deep ocean crustal rocks and the structure underlying islands as defined by geophysical data differs from that beneath deep ocean floors. The contrast suggests that special deep processes operate beneath oceanic islands. Most volcanic islands are confined to near spreading plate margins during their active period and their formation may be associated with the ocean floor spreading process²,³. Periodically, voluminous volcanic activity indicates exceptionally high rates of subcrustal convective heat transfer and mantle hot spots or plumes have been postulated as the sources of this activity¹.⁴.

In 1972 geoscientists from Dalhousie University and Lamont-Doherty Geological Observatory initiated a deep drilling programme into the oceanic crust with an 800 m hole into the island of Bermuda in the western Atlantic<sup>5,7</sup>. It yielded some 1,000 volcanic units approximately equally divided between thin altered tholeitic pillow lavas and lamprophyric intrusive sheets. Our second borehole in 1973 was located on the island of São Miguel, in the Azores, and an initial report is presented here of the 981 m penetration of a complex sequence of subaerial and submarine lavas, pyroclastics and volcanogenic sediments.

The Azores form a group of nine islands aligned in a NW-SE trending chain which transects the Mid-Atlantic Ridge (MAR) at 39° N (Fig. 1). The East Azores Fracture Zone and the MAR intersect at a triple junction<sup>8</sup> in the vicinity of the Azores and the region is marked with (i) a sharp change of trend of the MAR, (ii) the broadening of the ridge into an extensive platform<sup>8</sup> (Fig. 1) and (iii) an unusually high positive regional gravity anomaly<sup>10</sup>. The islands east of the MAR trend obliquely to the proposed plate margins and follow a pronounced bathymetric lineament (Fig. 1), the Terceira Rift<sup>8,11</sup> which extends through

parts of Graciosa, Terceira and São Miguel. Krause and Watkins<sup>8</sup> suggest that the Terceira Rift represents a secondary spreading centre trending towards Gibraltar which originated approximately 45 Myr ago.

São Miguel, the largest of the islands, lies near the eastern extremity of the chain 400 km from the MAR crest. Its surface geology is dominated by four large calderas, three of which have erupted during historic times12 and have produced an extensive blanket of trachytic pumice which covers much of the island<sup>13,14</sup>. Surface rocks range compositionally from alkali basalts to trachytes15,16 and are exceptionally potassic17. We selected a drill site on the gentle flanks of the volcano Agua de Pau which rises to a maximum height of 950 m above sea level and has a diameter at sea level of approximately 15 km. Its surface deposits consist of voluminous trachytic pumice and associated basaltic lavas intercalated with occasional trachytic extrusives. The last recorded eruption from the summit caldera in 1563 AD produced a cover of trachytic pumice and Walker and Croasdale<sup>18</sup> document four similar eruptive events during the past 4,600 years. Agua de Pau must therefore be considered to be currently dormant, as sporadic seismic activity beneath the caldera and a number of boiling hot springs on the flanks also indicate.

#### Stratigraphy and petrology of the core

Drilling began at 72 m above sea level, but only partial core recovery was possible in the first 148 m because of unconsolidated pyroclastic and mud flow deposits. Below this depth core recovery was virtually continuous to 981 m where drilling terminated following steam production. A synoptic core log is shown in Fig. 2.

Extrusive lavas constitute 72% of the drill core and occur in 140 separate flow units of 4.8 m average thickness and 2.5 m median thickness. Alkali basalts, hawaiites and mugearites predominate and only three trachyte flows (6% of total flow volume) were encountered. In the top 763 m of core a subaerial origin for the flows is suggested by (i) massive nature of flow centres, (ii) complete absence of pillow structures, (iii) intercalation of numerous pyroclastic units lacking any indications of aqueous reworking or stratification, (iv) development of some lateritic horizons, and (v) occurrence of thick vesicular and autobreceiated

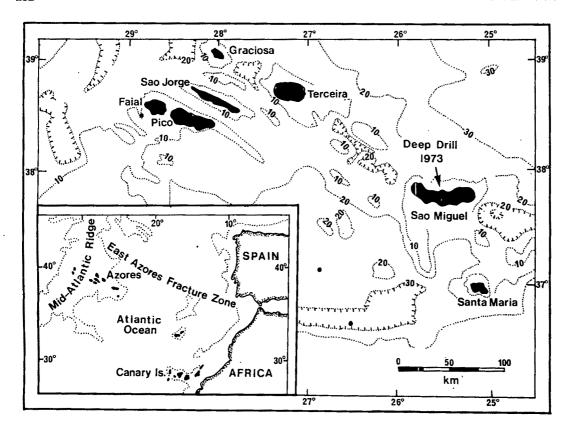


Fig. 1 Location map of Deep Drill 1973. Site coordinates 25° 31.4'W, 37° 48.9'N at 5 km from the caldera wall of Agua de Pau volcano, São Miguel. 1,000 m bathymetric contours are shown.

flow tops. Pillowed basaltic rocks with altered glassy margins were first encountered at 880 m where they are found interbedded with massive basaltic flows in a sequence devoid of pyroclastics.

In sharp contrast to the Bermuda drill core, intrusives constitute an insignificant fraction in São Miguel. Near vertical basaltic porphyry sheets in chilled contact with basaltic flows were encountered only at 662 m and 738 m.

Pyroclastic deposits, chiefly trachytic in character, account for 22% of the core and range from fine-grained tuffs to pumice deposits, agglomerates and mud flows. Indications of bedding are rare but when seen can be inclined steeply up to 30–40°. Steep depositional dips can also be seen in the recent ash-pumice deposits but these may not be an indication of tectonic tilting since contacts between flow units appeared to be near horizontal over the entire length of the core. Ignimbrite cooling units ranging in thickness from 6 cm to 3.8 m are common in the depth interval 262 m to 508 m. Only two such units have previously been described on the island<sup>17</sup>. Individual pyroclastic units are frequently trachytic in character at the base but grade upward into more basic compositions.

A 107 m thick igneous-sedimentary sequence marks the transition from subaerial to submarine volcanics. Basaltic breccias with a chloritic matrix (altered glass? or ash) are interbedded with massive flows in the upper part of the sequence (Fig. 3) and overlie 15 m of bedded coarse lithic sandstones. One 7 m bed consisting of angular basaltic fragments embedded in a matrix of black lithic sand (carbonate-quartz cemented) is strikingly similar to the black beach sands being formed along the Hawaiian coast line by flows entering the sea (Tilling, personal communication, 1974).

#### **Temperatures**

Bottom hole temperatures were monitored during drilling and temperature logs were measured at various intervals after drilling ceased (Fig. 4). The temperatures show 200° C water progressing upward from 550 m at the start to 290 m at the time of the last measurements. The boiling point was exceeded and steam erupted from the hole when the drill

rod was removed. The 200° C water would have boiled first when it reached the temperature-pressure boiling curve near 215 m depth. The steps in the last temperature profiles (for example at 110 m, 170 m, and 305 m) were produced by the convection water circulation in the hole because of loose ash sequences in which drill rods enlarged the hole considerably. Convection circulation in the hole is limited upward by tight basaltic and trachytic flows. It was also restricted above 305 m by casing.

Temperatures were nearly constant at 20° to 25° C to 100 m depth, a sudden jump to over 100° C occurred between 100 m and 175 m, then a uniform gradient of about 250° C km<sup>-1</sup> to a depth of 550 m and finally a very small temperature gradient of less than 10° C km<sup>-1</sup> to the bottom of the hole (Fig. 4).

In general, temperature profiles in hydrothermal areas show low vertical gradients in permeable rocks where the geothermal heat is carried upward by convection<sup>19</sup>. Impermeable rocks show high gradients since the heat must be carried through them by conduction. The region of this borehole is clearly not a normal hydrothermal system since the generally high porosity (and presumably high permeability) subaerial section above 550 m has a high gradient while the less permeable rocks below 550 m have a very low gradient. We suggest the following model to explain the observed temperatures.

The complete section penetrated by the hole probably does not permit vertical convection. Even though there are extensive permeable rocks (pyroclastics) above 550 m, circulation is restricted by frequent horizontal, impermeable horizons (flows). The lack of cellular convection is seen by the rapid change in measured temperatures with time when convection became possible in the borehole itself. Water flow parallel to the bedding (nearly horizontal), however, is probably not restricted in the permeable pyroclastic horizons. We suggest that there is hot water (205° C) flowing parallel to the bedding at about 550 m and water at 100° C flowing just under the impermeable layer at 110–120 m depth. The hot water probably moves down-dip in these horizons from its source in the upper regions of the volcano. There is no clear evidence for water flow in other

horizons. The low temperature gradient below 550 m suggests that there is no volcanic heat source directly beneath the drill site itself.

It is difficult to estimate the amount of hot water available in this area for geothermal power since no flow measurements were made. The hole erupted briefly with hot water and steam but was stopped after only 20 min, before any depletion of flow could be detected. The permeability of the core also has not been measured. Large volumes of 200°-210° C water may be available from near 550 m depth if the down-dip flow in the permeable horizons from higher on the volcano is rapid but flow tests are required to confirm this conclusion.

#### Age determinations

Palaeomagnetic measurements on lava flows in the core indicate that the subaerial, transition and subaqueous sequences are all normally magnetised. This probably indicates magnetisation during the Brunhes polarity epoch, although the possibility of remagnetisation during hydrothermal alteration cannot be discounted. If magnetisation does reflect geomagnetic field polarity the time scale of reversals of polarity of the Earth's magnetic field<sup>20</sup> provides an upper age limit of 0.69 Myr for the formation of the entire volcanic-sedimentary sequence.

Two samples were selected for radiometric dating by the conventional K-Ar method. The first of these, a slightly impure sanidine concentrated from a fresh trachytic flow located at 57 m depth has an apparent age of (117 $\pm$ 24)  $\times$ 10<sup>3</sup> yr (% K<sub>2</sub>O=6.7;  $^{40}$ Ar<sub>radiogenic</sub>/ $^{40}$ Ar=0.5). The second sample, a relatively fresh submarine lava from the 950 m level has an apparent whole rock age of (280 $\pm$ 140) $\times$ 10<sup>3</sup> yr (% K<sub>2</sub>O=2.7;  $^{40}$ Ar<sub>radiogenic</sub>/ $^{40}$ Ar=0.013). In other words, this

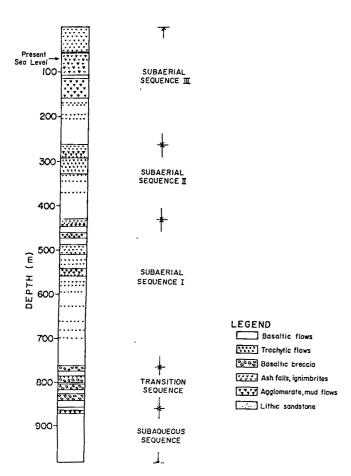


Fig. 2 Log showing major lithologic variations in the São Miguel drill core.

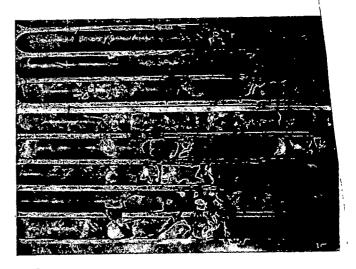


Fig. 3 Breccia from the transition zone consisting of angular basalt fragments of variable size in a matrix of coarse vulcanogenic sand and chloritic material. Basalt fragments show conspicuous leached margins. Length of core segments 0.6 m.

rock appears to have retained at least some radiogenic argon in spite of high ( $\sim 205^{\circ}$  C) temperatures encountered. The sample contained  $\sim 2.5 \times 10^{-6}$  s.c.c.  $g^{-1}$  atmospheric argon; an unusually high value perhaps indicative of exchange of gases between the lavas and overlying hydrothermal sources. We conclude from the radiometric and the magnetic data that the drill core spans a time interval of the order of a few hundred thousand years in the Brunhes normal polarity epoch and that the major vulcanism probably ended about 100,000 years ago.

Deep drilling on São Miguel has disclosed several important aspects of the island's geological evolution which are not evident from an examination of its surface geology. Three distinct subaerial eruptive sequences totalling 762 m in thickness were found to overlie a complex 107 m transition zone which documents the changeover from a subaerial to a submarine environment. Each subaerial eruptive sequence commenced with a quiescent phase of basaltic lava extrusion and was followed by increasingly explosive activity. Intercalated tuff beds increase in frequency and thickness and grade into a terminal phase of trachytic pumice deposition with rare trachyte extrusion. Compositional zonation within individual pyroclastic units further supports strong compositional zonation in the magma chamber at this stage. We suggest that each eruptive sequence signals the arrival of a fresh magma batch from depth in the mantle into a shallow magma chamber. Crystal fractionation and possibly assimilation at shallow depth would then yield increasingly differentiated products and an enrichment in volatiles; a combination which would in each case lead to increasingly explosive acidic eruptive activity.

#### Island subsidence

A surprising result of our experiment has been the depth at which submarine deposits were encountered. Subaerial or shallow marine conditions are found to 786 m below present sea level and indicate substantial subsidence of the island. Postglacial sea level rise since 18,000 BP can account for only 130 m of subsidence<sup>21</sup>. An evaluation of the glacial eustatic control of sea level for the earlier Pleistocene does not seem possible because of the largely unknown influence of tectonic crustal movements on sea level, but is not likely to be much greater. Paleomagnetic and radiometric dating of the core at younger than 0.69 Myr BP yields a minimum average subsidence rate of 0.1 cm yr<sup>-1</sup>. Other data on the vertical crustal motion in the

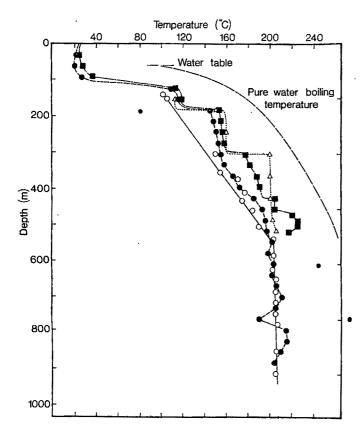


Fig. 4 Temperature measurements with maximum thermometers ( $\pm 2^{\circ}$  C) to 914 m during drilling; to 885 m with thermocouple ( $\pm 1^{\circ}$  C) between 1 and 2 h and again 4 days after termination of water circulation. Two days after further circulation maximum thermometer measurements were made to 518 m. O: temperatures at the bottom of the hole; temperature logs: ●: 26 August (1-3 h); ■: 2 September (4 d); △: 7 September (3 d).

Azores come from São Miguel's closest neighbour, Santa Maria, where a submarine sequence of basaltic volcanics and sediments of Miocene to Quaternary age is exposed22. This implies uplift for the island and suggests that the subsidence of Agua de Pau volcano is a local phenomenon.

The subsidence of ancient oceanic islands to form seamounts has been attributed to the thermal contraction of the lithosphere as it moves away from the spreading centre<sup>23-23</sup>, or to the vertical motion of the lithosphere as it slides over a bumpy asthenosphere<sup>26,27</sup>. In either case subsidence rates calculated from known spreading rates or theoretical models yield values of 0.01-0.02 cm yr<sup>-1</sup> which is an order of magnitude below those observed on São Miguel.

Detailed information on the vertical motion of other active volcanic islands is scarce. An analysis of tide-gauge measurements in the Hawaiian islands28 reveals recent subsidence rates of .0.5 cm yr<sup>-1</sup> for Hawaii, 0.2 cm yr<sup>-1</sup> for Maui, and stable conditions on Oahu. Decreasing subsidence rates correlate with an increase in the geological age of the islands and a decrease in intensity of their recent volcanic activity29. Ward30 has used raised shorelines and drilling data<sup>31</sup> from Oahu to demonstrate that Pliocene subsidence of the island was followed by uplift of 1.6 cm 1,000 yr<sup>-1</sup> during the Pleistocene.

São Miguel and adjacent Santa Maria exhibit a similar pattern. On Santa Maria, which is undergoing or has undergone emergence, neither historic eruptions nor any hot spring or fumarolic activity are reported. Volcanic activity has been dated to extend back 8.2 Myr<sup>32</sup>. The island is also furthest removed from active spreading centres such as the Terceira Rift. São Miguel, by contrast, shows rapid subsidence, active volcanicity, surface lavas dating from the historic era back to only 4.0 Myr<sup>12</sup>, and is situated on the currently active Terceira Rift.

The subsidence of an active volcano like São Miguel arises primarily from crustal loading with the addition of volcanic material. In Hawaii, Swanson<sup>33</sup> believes that simple isostatic adjustment adequately accounts for the observed subsidence. On a simple isostatic model the subsidence of 800 m observed on São Miguel requires the addition of a thickness of about 1 km of subaerial volcanic material to the island and will result in an increase of average elevation of 100-200 m. When a volcanic island becomes extinct, such as Santa Maria, erosion will result in isostatic rebound or uplift. If the top of the submarine section was 800 m below sea level when the island was active, at least 1,000 m has been eroded with a general reduction of elevation of 100-200 m.

Our temperature measurements suggest that the northern flank of Agua de Pau represents a promising prospect for future geothermal exploration. Bottom hole temperatures are well within the range of productive geothermal fields34 and occur at profitable depth. The thick subaerial volcanic sequence with its high proportion of pyroclastics and thick scoriaceous flow tops provides high porosities and possibly also permeabilities which make high flow rates probable. Further investigations to determine total hot water reserves, possible extraction rates, and the chemistry of the steam and water in this and other wells will be necessary for more specific evaluations. Seismic profiling to evaluate trap conditions near our borehole would seem a sensible next step.

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# Observation of tissue metabolites using <sup>31</sup>P nuclear magnetic resonance

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<sup>31</sup>P NMR spectra of intact biological tissues can now be observed. The use of the spectra to study the course of reactions within the tissues is illustrated by experiments on muscle and its glycogen particle fraction.

Although phosphorus NMR has only 1/16 the sensitivity of proton NMR, it is attractive because studies may be done in aqueous solution and the spectra are relatively simple because of the small number of different chemical environments in which phosphorus atoms are found. In addition, the chemical shift range is much larger for phosphorus than for protons.

As a result of considerable instrumental improvements achieved recently in both the magnitude of the magnetic field and the sensitivity of the detection we are now in a position to study <sup>31</sup>P resonances in a large variety of systems at concentrations found naturally in biology, using Fourier transform and impulse response techniques2. Useful spectra can now be obtained of systems varying in complexity from solutions of purified enzymes to intact tissues.

#### Phosphate resonances

At the high magnetic field strength employed (7.5 tesla) the <sup>31</sup>P resonances of a large number of biologically important phosphate-containing compounds can be resolved. The chemical shifts of the phosphate groups are in the range of about 30 p.p.m., and many sugar phosphates and glycolytic intermediates can be resolved. Furthermore, the state of ionisation of the phosphates and their interaction with metal ions, such as Mg<sup>2+</sup>, affect the positions of the resonances. Figure 1 shows <sup>31</sup>P spectra of a mixture of compounds recorded at various pH values, the individual resonances having been assigned by observing the spectra of the individual components.

The resolution of the resonances from various phosphoruscontaining small molecules allows rapid assay of the components of mixtures of these molecules. Measurements are carried out without destruction or dilution of the sample, and provide a method of monitoring turnover and interconversions of these molecules in organelles.

In the glycogen particulate fraction isolated from rabbit muscle, covalent enzyme regulation has been exhaustively studied<sup>3,4</sup> and transient phosphorylation of phosphorylase b has been shown to be the principal trigger for glycogen breakdown. The enzymes concerned are also regulated by small ligands. Therefore, knowledge of concentrations of these regulators at any point in the transient covalent activation is important for full understanding of the control mechanism<sup>5</sup>. Figure 2 shows the turnover of the phosphorus containing

ligands during a typical transient activation, obtained from a series of <sup>31</sup>P NMR spectra, recorded on a single sample. The active form of phosphorylase immediately catalyses glycogen breakdown, leading to the production of glucose-1-phosphate and so (by phosphoglucomutase activity) to glucose-6-phosphate. The production of glucose-6-phosphate is concomitant with phosphate utilisation. During the phosphorylation of phosphorylase b, ATP is converted to ADP. Because of the presence of adenylate kinase, which catalyses the reaction 2ADP 

ATP + AMP, and of AMP-deaminase, which catalyses the reaction AMP-IMP+NH<sub>3</sub>, the ADP initially formed is depleted and IMP—not distinguishable from AMP by  $^{31}P$  NMR —is produced. The instability of the nucleotide levels indicates that the system is not a very good model for extrapolation to the in vivo situation.

The assay technique outlined above has also been used to estimate the separate activities in a crude extract of homogenised rabbit muscle by following the sequential production of glycolytic intermediates. The mass action ratios for some of the

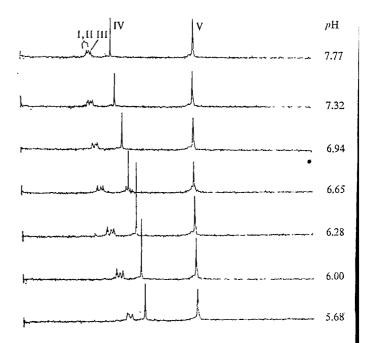


Fig. 1 <sup>31</sup>P NMR spectra of a mixture of fructose-1, 6-diphosphate (I, II), IMP (III), inorganic phosphate (IV), creatine phosphate (V) at various pH values, recorded at 129 MHz. No buffer present; total phosphate concentration 60 mM. Sweep width 5 kHz, pulse interval 2 s, 200 scans. Spectrum recorded without proton irradiation recorded without proton irradiation.

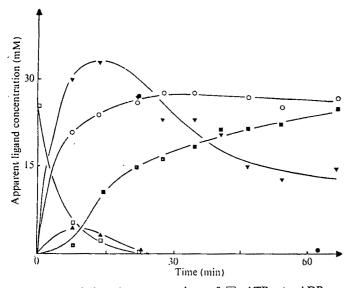


Fig. 2 Variations in concentrations of □, ATP; ▲, ADP; ○, AMP and IMP; ■, glucose-6-phosphate; and ▼, inorganic phosphate during flash activation of phosphorylase in glycogen particles. (27 mM ATP, 25 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 50 mM triethanolamine, 100 mM potassium chloride, 1 mM EDTA pH 6.9). ATP was added at time zero. Sweep width 5 kHz, Pulse interval 3 s, 50 scans. Spectrum recorded without proton irradiation. Differential saturation of the resonances can lead to small errors in the relative concentrations of ligands as measured from the areas under the peaks. The measured concentration of any ligand, however, is always a fixed proportion of its true concentration.

individual reactions have been deduced from the results obtained. The technique has also rapidly revealed the presence of contaminant activities in supposedly pure enzymes.

#### Metabolites in whole muscle

Our attempts to follow enzyme activities in intact tissues using phosphorus NMR have, to date, been principally concerned with observations on muscle from the hind leg of the rat. Figure 3 shows the <sup>31</sup>P spectrum of an intact, relaxed muscle, freshly excised from a rat killed by etheration. Assignments are made on the basis of the pH titrations mentioned in section 1. Part of the 'sugar phosphate' peak is attributed to

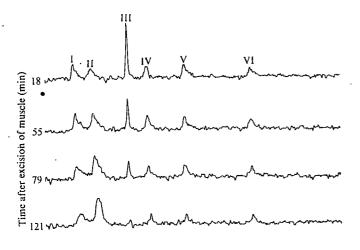


Fig. 3 <sup>31</sup>P NMR spectrum of an intact muscle from the hind leg of the rat recorded at 129 MHz, without proton irradiation. Temperature 20° C and pulse interval 16 s. Peak assignments: I, sugar phosphate and phospholid; II, inorganic phosphate; III, creatine phosphate; IV, γ ATP; V, α ATP; VI, β ATP. The times are the midpoints of the 50 scan spectral accumulations (referred to excision time as zero). The muscle was bathed in a minimum volume of calcium-free Locke ringer.

phospholipid as there is a broad peak of identical chemical shift in the phosphorus spectrum of debris from muscle extracted with aqueous buffer. Assays on this aqueous extract record a concentration of about 1 mM for glucose-6-phosphate. The frequency of the inorganic phosphate resonance defines the apparent pH of its environment (7.1 in this spectrum) and the frequencies of the ATP peaks correspond to those for the Mg<sup>2+</sup>-ATP complex at the phosphate pH. (The frequency of the creatine phosphate resonance is independent of pH around 7.) In vitro studies of Mg2+ binding to ATP show that there are large shifts (2-3 p.p.m.) in the  $\beta$ - and  $\gamma$ -phosphate resonances on complex formation. These shifts show that the ATP observed in muscle is almost entirely complexed to magnesium ion. Changes in ionic strength also produce measurable spectral shifts, but these are much too small to alter our assignments for the intact muscle spectrum.

The signal peaks of the muscle phosphates are broader than those for the corresponding compounds free in solution, and the inorganic phosphate peak is markedly broader than the ATP or creatine phosphate resonances. The explanation for this phosphate broadening cannot lie in a disturbance of magnetic field homogeneity caused by the nature of the sample as the width of the creatine phosphate signal gives an upper limit for

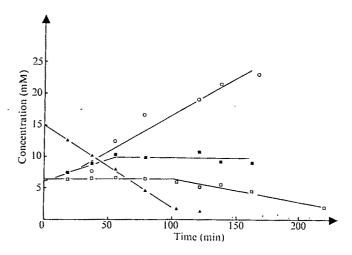


Fig. 4 Variation of phosphorus metabolite levels in an intact rat leg muscle with time after excision. The integrals of spectra shown in Fig. 3 are plotted in this graph. ○, Inorganic phosphate; ▲, creatine phosphate; ➡, sugar phosphate and phospholipid; □, ATP. An absolute concentration scale was established by running a standard sample of 10 mM phosphate in the same conditions as the muscle.

the field inhomogeneity. Inorganic phosphate has a pK at 6.9 whereas the nearest pK for creatine phosphate is at 4.6. The frequency of the former resonance is therefore strongly pH dependent around pH 7 whilst that of the latter is pH independent in this region. It seems likely that the large width of the phosphate line may be due to a distribution of pH within the muscle, that is to some partial compartmentation of inorganic phosphate. The pH range that would account for the linewidth is about 0.5 pH units.

We have measured the approximate concentrations ( $\pm 10\%$ ) of phosphorus metabolites in the intact muscle by integration of the absorption spectrum, using a sample of known concentration as a standard after taking care to avoid saturation of the resonances. There is some variation from muscle to muscle, and the creatine phosphate: phosphate ratio proves to be a sensitive index of the degree of stimulation of the rat muscle before the animal's death, that is a high creatine phosphate: phosphate ratio in rats killed without excessive stimulation. We have measured the concentrations of 'sugar phosphate', inorganic phosphate, creatine phosphate and ATP at intervals between 16 and 170 min from excision of the muscle (Fig. 4). Extrapolation along the time axis shows the metabolite levels

at the time the muscle was excised to be: sugar phosphate+ phospholipid, 6 mM; inorganic phosphate, 6 mM; creatine phosphate, 15 mM; and ATP, 6.5 mM.

Creatine kinase maintains the ATP level constant at the expense of creatine phosphate until all the latter substrate has been used up, demonstrating the ability of the kinase to buffer the muscle ATP concentration. Ageing of the muscle is accompanied by a fall in pH which is monitored by changes in the frequencies of the phosphate and ATP resonances. The pH at the start of data accumulation is 7.1, and after 160 min it has fallen to 6.2. Acid accumulation seems to accelerate at the time when the creatine phosphate concentration falls to zero and glycolysis presumably begins.

These results can only be obtained with intact muscle. Samples which are even slightly lacerated during handling have only an inorganic phosphate peak in the phosphorus spectrum. Breakdown of organic phosphates by phosphatases is assumed to have occurred in the damaged muscle.

#### Scope

On the basis of these experiments and other data we have obtained from a variety of biological systems, we believe that <sup>31</sup>P NMR can yield general information about metabolite levels, turnover, interactions and compartmentation. As it is

also possible to distinguish between signals from extrace and intercellular materials, in favourable cases the stud certain transport processes is now also possible. The delines of unusual pathways, the detection of possible new in mediates and the presence of bound nucleotides also se within the scope of the method. To what extent the kind structural information that is beginning to emerge from solution studies can be extended to the study of intercellu components remains to be determined.

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# letters to nature

### Degeneracy effects on neutrino mass ejection in supernovae

THAT type II supernovae apparently occur predominantly in stars significantly more massive than the Sun has been known1 for some time, although a precise stellar mass range for these cataclysmic events can not be defined observationally. Recent theoretical interpretations<sup>2-4</sup> of diverse observational data seem, however, to indicate independently that the masses are significantly in excess of  $8M_{\odot}$ . Furthermore, observational evidence may exist that stars with masses below  $6M_{\odot}$  cease nuclear burning before a critical supernova configuration evolves. If this is the case, the total supernovae contribution from the stars of lower mass is negligible and theoretical difficulties6 associated with such events are resolved. The circumstantial evidence for massive  $(>8M_{\odot})$  supernova progenitors is thus reinforced, although problems arise in understanding the supernova mechanism.

Attempts to explain supernovae in massive stars theoretically have centred on either nuclear fuel detonation? or neutrino energy deposition<sup>8</sup> in the infalling envelope during dynamic stellar collapse. The former mechanism does not release sufficient energy (ref. 8 and Z. K. Barkat, J. C. Wheeler, J. R. Buchler and G. Rakavy, unpublished) to account for the type II explosion. The published results9 on the latter are in dispute10.11 but tend to be negative with respect to the required mass ejection. Recently it was suggested12 that the neutrinomatter coupling may be enhanced considerably through coherent scattering from nuclei. Subsequent hydrodynamic computations<sup>13</sup> required a fivefold increase in the theoretically estimated cross section for effective neutrino mass ejection. As coherent effects diminish at the high average escaping neutrino energy<sup>8</sup> (~50 MeV), the results are not very encouraging. Thus the posited mechanisms for massive stellar explosions seem inadequate.

The strong circumstantial evidence for massive supernovae progenitors, however, requires a critical re-evaluation. The comments in this communication will focus on the neutrino transport mechanism. All extant computations impose the condition of a zero chemical potential on the neutrinos. Arbitrary variations in the neutrino numbers result as a photon-like blackbody spectrum is maintained. Neutrinos and antineutrinos contribute to the total lepton number, however, and therefore can not be created or destroyed arbitrarily. Indeed I shall argue that the arbitrary zero chemical potential condition suppresses leptonic neutrino transport characteristics that may lead to supernova mass ejection.

My study was limited to high densities (>  $10^{11} \, \text{g} \, \text{cm}^{-3}$ ) where the diffusion approximation to neutrino transport obtains11. In this approximation the Boltzmann transport equation transforms by way of the Lorentz-Enskog method into a relationship between the nonisotropic neutrino intensity and the gradients in temperature and chemical potential. Appropriate integration over the neutrino spectrum (the canonical electron scattering opacity14 was assumed) then yields the Rosseland mean approximations for two neutrino fluxes, one for energy and one for particle number15.

Comparison of the resulting coupled energy/particle diffusion equations with that of the usual diffusion approximation16 indicates that the latter suppresses two potentially significant leptonic effects: energy transport caused by chemical potential gradients, and variations in the efficiency of thermal transport with particle number concentration (that is, chemical potential variations). As they have not been accounted for in the extant computations, the question arises of their significance for neutrino supernovae.

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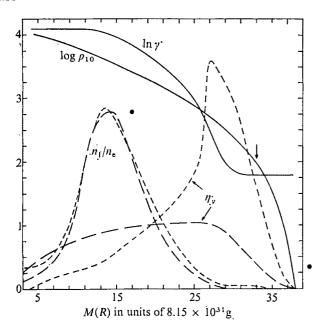


Fig. 1 The distribution of diffusion parameters with the total mass interior to a given radial point in the collapsing structure after diffusion has progressed for 0.445 ms. The parameters shown include the temperature  $(kT = \gamma m_e c^2)$ , the density  $(\rho = 10^{10} \rho_{10})$ , the ratio of the chemical potential to  $kT(\eta_{\nu})$ , and the ratio of the neutrino lepton number to that of equilibrium matter electrons. The arrow indicates where the gravitational free-fall time scale equals the elapsed time. — decoupled.

For a quantitative answer, the degree of neutrino particle/antiparticle interaction must be determined. Rapid interaction on transport time scales gives neutrino/photon equilibrium and will be referred to as the coupled transport mode. When no interaction is possible the decoupled mode obtains. Coupling may be mediated either through particle/antiparticle annihilation or through equilibrium in both urca reactions:

$$v_{e}[(Z, A), (Z+1, A)] e^{-1}$$
 and  $\overline{v}_{e}[(Z+1, A), (Z, A)]e^{+1}$ .

Electron pair production should dominate in neutrino annihilation as one and two photon processes cannot contribute17. Hence coupling will decrease with electron degeneracy. For thermalised neutrinos, coupling may be impossible on energetic grounds alone in electron-degenerate outer regions of collapsing supernova configurations, and a transition from coupled to decoupled transport may be possible between the hot central condensation and the collapsing outer envelope. The details of such a transition were ignored in this preliminary study, and only the extremes of total coupling and decoupling were investigated.

Neutrino diffusion was studied in a supernova ambience to determine the leptonic effects in transport. Coherent scattering by nuclei was neglected; thus muon neutrinos escaped essentially without interacting. The investigation used a stationary collapsed stellar density structure that corresponds to published results9 of hydrodynamic collapse at a given instant. The initial temperature structure was essentially that of Fig. 1 and the chemical potential was initially set to zero throughout. The shock energy and lepton number deposition was approximated by means of source terms using published shock structure estimates11. Ninety per cent of this energy was assumed to escape in the form of muon neutrinos<sup>9</sup>. The electron neutrino lepton number increased at the rate of matter transport through the shock to higher density with lower electron number<sup>18</sup>. The two equations for diffusion of energy and of particles were solved numerically for the coupled and decoupled transport modes. Fluxes were allowed to escape without interaction for densities below 1011 g cm -3.

The resulting configuration after 0.445 ms of evolution is shown in Fig. 1. A striking result for both transport modes is the concentration of neutrino lepton number at the shock front (its value was initially zero). The arrow indicates the point at which the hydrodynamic free-fall time equals the elapsed time of diffusion. The hydrodynamic time scale rapidly diminishes at the higher densities at which the neutrino lepton number concentrations occur. The neutrinos are therefore virtually trapped on hydrodynamic time scales and will fall with the matter. The rate of neutrino lepton number generation at the shock front is therefore greater than that assumed in the present static investigation. The actual hydrodynamic collapse of the matter will aggravate the chemical potential build-ups obtained.

The distributions of chemical potential for both diffusion modes is significantly different from zero. Pair energy transport inhibits the potential's growth in the coupled case. The large build-up for the decoupled case may indicate steep chemical potential gradients opposite to those of temperature if a transition from coupling to decoupling occurs.

The modifications on the hydrodynamics of supernovae that may be implied by these results are difficult to predict. An order of magnitude estimate of the energetic differences that could arise was obtained by continuing the above computations to 7.5 ms. For purposes of comparison with extant supernova computations a similar diffusion study was carried out for coupled transport subject to a fixed zero chemical potential condition. The net energy and lepton number escape from th. structure for the three different cases were as shown in Table 1.

Table 1 Net energy and lepton number escape						
Energy (erg)	Lepton number					
$8.6 \times 10^{51}$	$(N_{\text{Avg}}\text{-g})$ 2.7×10 <sup>32</sup>					
$2.7 \times 10^{51}$	$3.3 \times 10^{32}$					
$3.2 \times 10^{51}$						
	Energy (erg) 8.6×10 <sup>51</sup> 2.7×10 <sup>51</sup>					

The case in which a zero chemical potential is imposed most closely resembles the decoupled mode as far as energy is concerned. If a transition from coupled to decoupled diffusion occurs in actual supernovae, energy may accumulate at the transition point because of differences in diffusion rate. The above energy difference of  $\sim 5 \times 10^{51}$  erg is more than sufficient to give a type II supernovae. The possibility of such an accumulation of energy in the outer regions needs to be carefully considered.

These preliminary results indicate that the effects of lepton conservation may be significant in supernova neutrino transport. The validity of the diffusion approximation becomes questionable at lower temperature and density because of the long mean free paths of low energy neutrinos. The portion of the equilibrium neutrino spectrum thus affected is, however, relatively small over most of the collapsing supernova structure. Thus the general features of leptonic transport should be reflected in the results presented. The large magnitude of leptonic effects in diffusion shows that they cannot be ignored a priori. An extension of the multi-energy group transport approximation9 to allow for these effects is desirable.

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### Measurement of atomic oxygen in the lower ionosphere using a rocket-borne resonance lamp

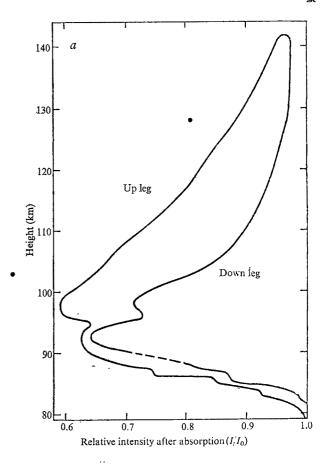
Atomic oxygen is an important constituent of the mesosphere and lower thermosphere because of its role in the excitation of the O I( $^{1}S_{0}^{-1}D_{2}$ )· airglow emission at 557.7 nm (ref. 1) and because of its involvement in the positive and negative ion chemistry of the lower ionosphere<sup>2</sup>. In particular it destroys negative ions<sup>3</sup> and can break reaction chains believed to produce water cluster ions below about 85 km (ref. 4). There is also uncertainty about the degree of dissociation of oxygen in the thermosphere<sup>5,6</sup> and atomic oxygen is involved in many reactions with neutral hydrogen/oxygen compounds in the mesosphere<sup>7</sup>.

Various experimental techniques have been used to measure the concentration of atomic oxygen [O] (refs 8-12) but most of these have serious limitations or uncertainties. We report direct measurements of night-time concentrations of atomic oxygen in the ground state, O ( $^{3}$ P), in the mesosphere and lower thermosphere by a new technique. Values of [O] from  $3 \times 10^{7}$  to  $5 \times 10^{11}$  cm<sup>-3</sup> have been found at heights between 79 and 142 km. The experiments were rocket-borne, and used resonance fluorescence and absorption techniques at the O I( $^{3}$ P- $^{3}$ S<sub>1</sub>) triplet, 130.20, 130.47 and 130.58 nm.

O I radiation was produced in the rocket payload by a resonance lamp of novel design. It consisted of a sealed coaxial radiofrequency discharge tube within which molecular oxygen was produced by a temperature controlled chemical source, balanced by a getter. The oxygen pressure was adjusted to give minimal self absorption of the 130 nm radiation by atomic oxygen in the discharge. This was determined by laboratory measurements of the relative intensities of the components of the triplet, which dominated the spectral output of the lamp at 10<sup>13</sup> photons s<sup>-1</sup> sr<sup>-1</sup>. The lamp also radiated at H Lyman-α (121.6 nm), at the O I (<sup>5</sup>S- <sup>3</sup>P) 136 nm lines, and at longer wavelengths.

The emission from the lamp was concentrated by a concave cylindrical mirror into a flat beam in the plane perpendicular to the rocket axis. A small part of the beam was reflected back by a corner reflector deployed on a boom and was detected by an ion chamber to measure absorption by ambient atomic oxygen over a 40 cm path. The ion chamber has a  $\text{CaF}_2$  window and NO gas filling and was operated at a gas gain of about 600. Radiation at 130 nm was the only emission from the lamp within the bandpass of the detector (123 to 134 nm).

A photomultiplier was used to count photons re-emitted by the ambient oxygen atoms due to resonance fluorescence under



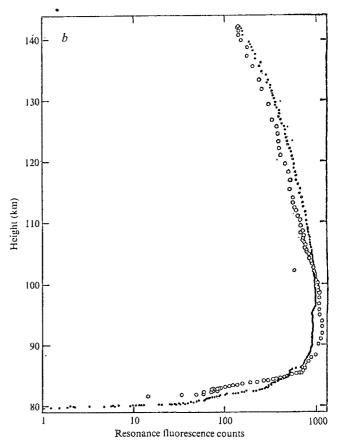


Fig. 1 a, Absorption of O I (130 nm) radiation over a path length of 40 cm through ambient atomic oxygen as a function of height for the complete rocket flight. b, Simultaneous measurements of the intensity of resonance fluorescence emission at  $\lambda = 130$  nm from ambient atomic oxygen as a function of height.  $\bullet$ , Up leg;  $\bigcirc$ , down leg.

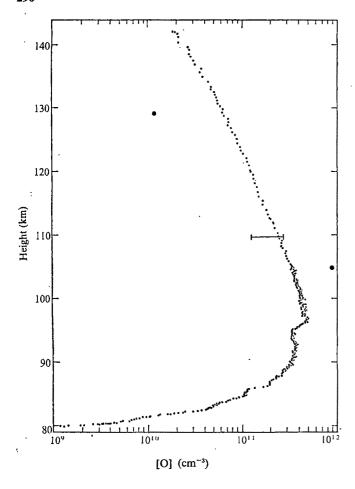


Fig. 2 Atomic oxygen concentrations calculated from the absorption and resonance fluorescence results as a function of height (up-leg data only). The error bar indicates the maximum range of uncertainty in the absolute scale.

irradiation by the ultraviolet beam. The lamp intensity was square wave modulated at 240 Hz so that phase-sensitive detection could be used to reject background signals due to airglow. A CaF<sub>2</sub> window was again used to suppress Lyman-a background. The field of view of the photomultiplier excluded all payload components.

The concentration of atomic oxygen which was required to produce a given degree of absorption was determined in the laboratory by measuring across a flowing afterglow in which known atomic oxygen concentrations were produced. This allowed absolute values of [O] in the ionosphere to be found from the absorption experiment in flight.

The resonance fluorescence experiment was not used to obtain independent absolute measurements of [O] because knowledge of all factors affecting the optical efficiency of the experiment would have been required, resulting in a relatively large uncertainty. But absolute values obtained from the absorption experiment are independent of these factors and were used to calibrate the resonance fluorescence for [O] values in the approximate range  $2 \times 10^{10}$  to  $5 \times 10^{11}$  cm<sup>-3</sup>. This calibration indicated some non-linearity in the resonance fluorescence count response at high count rates which was consistent with the known characteristics of the electronics as measured in the laboratory. At lower count rates a linear dependence of count rate on [O] was obtained and used to extrapolate the calibrations to [O] values below the range of sensitivity of the absorption experiment.

Two payloads, each including the above experiments and a Langmuir probe, and longitudinal and transverse magnetometers, have been flown on Petrel rockets. Rocket number P174H was launched at night and P173H at dawn. Each flight was followed within 20 min by a second Petrel rocket carrying a support payload to measure atomic oxygen using resistive silver film sensors<sup>5</sup> and electron density by Faraday rotation and Langmuir probe. Useful results were obtained from all four flights.

This letter deals solely with atomic oxygen concentrations from P174H. The flight was made from South Uist, Scotland (57° 20'N, 7° 20'W) at 2237 UT on April 1, 1974, under magnetically quiet conditions. It reached an apogee of 142 km and was spin stabilised. The experiments were all operating from 64 km upwards. The trajectory was determined from Döppler slant range measurements and radar tracking, and is accurate to better than  $\pm$  0.5 km in height.

The dependence of the measured absorption on height is shown in Fig. 1a. This may be compared with the counts from the resonance fluorescence in Fig. 1b, each point being the average of six counts sampled at 240 Hz. The derived atomic oxygen concentrations are shown in Fig. 2 for the up-leg data only.

In calculating the effective length of the absorption path in flight, molecular flow and complete recombination of oxygen atoms at surfaces have been assumed. If the surface recombination were low a stagnation zone of high [O] in part of the absorption path might cause an overestimation of the ambient concentration. In Fig. 2 this unlikely case is represented by the minimum density end of the error bar which indicates the range of uncertainty in the absolute [O] scale.

The atomic oxygen concentration was below  $3 \times 10^7$  cm<sup>-3</sup>, the lowest detectable value, until 79.6 km was reached. Incre an extremely rapid monotonic rise by a factor of 40 in 0.4 km occurred, which has been omitted from Fig. 2 to avoid undue contraction of the [O] axis. (The omitted data can be seen in Fig. 1b below about 10 counts.)

Between 80 and 97 km, [O] increased and the measurements showed departures from a smooth variation. These occurred between 82.5 and 83.5 km, between 84.8 and 87 km and between 93 and 96 km. These perturbations were detected by both the absorption and resonance fluorescence experiments on the up and down legs of the flight at the same heights. They are, therefore, considered to indicate fine structure in the variation of [O] with height and not an instrumental effect.

Above the maximum [O] at 97 km the fall off became approximately exponential with a scale height of  $14.9 \pm 0.6$  km.

On the down leg the resonance fluorescence response was lower and the absorption considerably lower than on the up leg, except at the down-leg maximum near 93 km where both responses were similar to the up leg. A reduced response was expected on the down-leg because of wake effects.

The rocket velocity was high enough to cause serious loss of resonance due to the Döppler effect if there were a significant component of the velocity along the ultraviolet beam. The beam geometry had therefore been chosen to make this error negligible on the up leg. But on the down leg, roll modulation was observed which may be attributed to the Döppler effect as predicted.

The concentrations shown in Fig. 2 are derived from up leg data only, for which attitude corrections are expected to be small except near apogee.

The results have been produced by analysis of ultraviolet paper records of the analogue data. We intend to publish fuller details of the experiment and more precise results from computer analysis of digital records later.

This work was part of a joint programme of D-region research by the Appleton Laboratory of the Science Research Council, and the University College of Wales, Aberystwyth; we thank Professors W. J. G. Beynon and N. D. Twiddy, Drs W. C. Bain and L. Thomas, and Mr E. R. Williams for advice. The rocket payload was instrumented by K. Slater and G. E. A. G. Barnett (Appleton Laboratory). The resonance lamp was designed at York University, Toronto, Canada, and developed and constructed by Intra-Space International Inc., Downsview, Canada. The lamp was calibrated at the UCW

which also provided the support payloads. Assistance with analysis of flight data was provided by Md Iqhbal Ahmed, Commonwealth Academic Staff Fellow, Andhra University, Waltair, India.

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# Thermospheric winds from laser tracking of sodium clouds

NEUTRAL wind measurements in the thermosphere have been made on many occasions using ground based photographic¹ or photometric observations² of artificial clouds released from rockets. These clouds are visible either through a chemiluminous reaction, such as with trimethyl aluminium (TMA) releases, enabling night-time observations to be made at altitudes between 80 and 150 km or through resonance emissions of sodium, lithium, barium or aluminium oxide when clouds are illuminiated by sunlight, enabling twilight or daytime observations to be made above about 80 km.

To measure night-time winds at all heights above 80 km, in particular above 150 km, the normal limit of a TMA trail, we

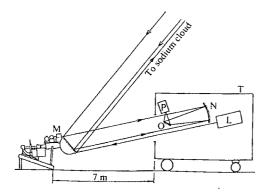


Fig. 1 Steerable laser radar. L, Rhodamine 6G flashlamp-pumped dye laser; M, steerable plane mirror (0.6 m × 0.4 m), N, O, Newtonian receiving telescope (aperture diameter 0.3 m), P, photomultiplier, processing and recording electronics; T, equipment trailer.

Table 1 Parameters of the steerable laser radar as used for rocket P146A

Transmitter	
No. of transmitted photons per pulse Laser pulse repetition frequency Wavelength Linewidth	1.4 × 10 <sup>17</sup> 5 Hz 589.0 nm 3 pm
Beam divergence Receiver	2.5 mrad
Overall efficiency Beam divergence	0.04 5 mrad
Raster scan Size Time for one raster	20° × 20° or 20° × 40° 100 s or 200 s

have built a new mobile laser radar system which is used to track sodium clouds released from rockets. This letter reports the operation of the system during the United Kingdom high latitude rocket campaign at Andenes, Norway, in October and November 1973 and gives the wind profile obtained during one of the four successful flights.

The steerable laser radar (Fig. 1) was developed at the Appleton Laboratory from a fixed system previously used for studying the natural sodium layer by means of resonance scattering of a tuned laser beam<sup>3</sup>. The plane mirror, which is used to scan the laser beam around the sky, rotates about two axes, allowing the system to cover nearly half the sky. The scan is controlled

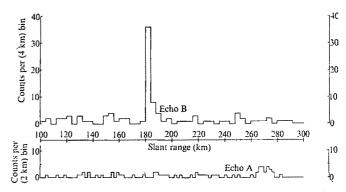


Fig. 2 Echoes from the sodium trail. Echo A (pulse No. 1007) is at 174.5 km altitude, 288 s after launch. Echo B (pulse No. 2885) is at 141 km altitude, 667 s after launch. The probability that the dark current and background signals, which should obey Poisson statistics, could produce a noise signal equivalent to these echoes is very small.

remotely in either manual or automatic modes. In the latter, a selected portion of the sky can be scanned in a raster. After each laser pulse, the photons detected by the photomultiplier are counted as a function of time delay and therefore slant range. The counts from each of 100 successive slant range intervals (where the intervals can be chosen to correspond to 0.5, 1, 2, 4 or 8 km), a time code and the angular coordinates of the scanning mirror are recorded in a suitable format on a digital cassette recorder for subsequent computer analysis. In addition analogue signals are reconstituted from the digital data to provide a quick-look record on an ultraviolet oscillograph for immediate analysis and also a real time display on a cathode ray tube of intensity of echo as a function of slant range for each laser pulse. The location of the detected cloud within a given raster is stored and displayed continuously on a second cathode ray tube. This real time information on position, intensity and slant range of the cloud echoes enables the operator to optimise the raster location and the slant range settings of the recording system for subsequent rasters.

The system was deployed at Skibotn (69° 22'N, 20° 18'E) about 170 km from the rocket range at Andenes (69° 18'N,

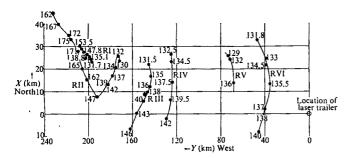


Fig. 3 Ground projection of sodium trial. I to VI, Raster number; altitudes (km) are marked. The coordinates and altitudes shown are means, derived from analysing between 20 echoes (Raster I) and 160 echoes (Raster IV) per raster.

16° 02′E). Sodium thermite burners which would release a sodium cloud in the form of a trail were launched on four Petrel rockets (P100A, P101A, P145A, P146A) and as part of the payload of one Skylark rocket (SL1123). Rockets P100A (October 14, 1973) and P101A (October 17, 1973) were used to provide initial tests of the system for developing operational techniques. SL1123, followed by P145A and P146A were launched on the night of November 16/17, 1973 at intervals of approximately 1 h during a strong auroral substorm. Four of the five sodium trail releases were acquired and tracked, the one failure (P101A) being because of a partial rocket malfunction which caused a very low apogee.

The data presented here were obtained from P146A which was launched at 0101:02 ut on November 17, 1973, into the recovery phase of an auroral substorm. The sodium trail started near 130 km on the up leg, continued over apogee (175 km) and rather weak outgassing of sodium vapour continued to about 160 km on descent (the apogee and descent portions of the trail appeared from the laser echoes to be optically thin). The laser radar was operated for 35 min after the trail was released with the system parameters given in Table 1. 1,500 echoes from the trail were recorded from 10,000 laser pulses. Figure 2 shows two echoes. From the intensity of echo B at an altitude of 141 km it was deduced that the trail was probably optically thick. The return from 174.5 km (echo A) is from a well diffused and optically thin region near apogee.

Figure 3 shows the projection of the sodium cloud on to the Earth's surface (geocentric coordinates) as observed with the first six rasters. An average wind profile, Fig. 4, was deduced by following the projected ground track of various altitudes between the rasters.

Strong eastward and southward wind components above 130 km seen during the experiment are probably consistent with those to be expected from a combination of ion drag accelera-

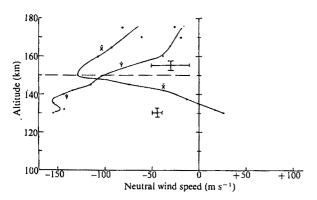


Fig. 4 Average wind profile for P146A.  $\dot{X}$ ,  $\dot{Y}$ , Northward and westward components of velocity. Error bars indicate the maximum errors ( $\pm$  3 standard deviations) in the two regions above and below -150 km.

tion and extensive heating in the auroral thermosphere during the auroral substorm of magnitude— $500\gamma$  (nT) at Andenes, earlier in the night. The significance of these results regarding thermospheric morphology during auroral substorms will be studied using the results of all three rockets (SL1123, P145A, (146A) launched during the substorm.

During this campaign the greatest altitude at which echoes and wind data were obtained was the apogee of the Skylark (215 km). From the intensity and duration of the signal received at 215 km, we estimate that the laser radar system we used for these experiments can obtain wind profiles from sodium clouds at over 300 km altitude, the predicted design limit. This technique gives a considerable extension of the upper altitude limits at which night-time wind measurements can be made from rocket releases.

We thank the staff of the Auroral Observatory, Trömso, for assistance in installing and maintaining the laser radar trailer at Skibotn. This was a collaborative project between University College, London, and the Appleton Laboratory of the Science Research Council. The rocket launches and the development work were supported by the Science Research Council. Mr E. Hammond helped to construct and operate the laser radar.

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# Atmospheric halocarbons and stratospheric ozone

ODD chlorine<sup>1</sup> is a more potent catalyst for the destruction of ozone than is odd nitrogen<sup>2</sup>. Molina and Rowland<sup>3</sup> warn that chlorine released during the photochemical destruction of fluorocarbons (FCCs) constitutes a potentially serious threat to the integrity of the stratospheric ozone layer.

In the original investigations of the atmospheric distribution of FCCs (refs 4-7) these compounds were sought because of their unique usefulness as easily detected and unequivocal anthropogenic markers. Their presence in an air mass at elevated levels indicated its recent passage over an urban industrial region. The discovery of other halocarbons, accidentally revealed by the analysis, although scientifically interesting, was not the prime motive. So the literature on this topic gives the unintended impression that the FCCs are the predominant chlorine carriers in the atmosphere. In fact they are less abundant than other halocarbon classes and their presence might not have been discovered but for the exquisite sensitivity of the analytical method used.

Table 1 lists the observed abundances of the halocarbons at Adrigole in western Ireland in June and July 1974 in air coming from the west and in mid Atlantic aboard the research vessel Meteor in its cruise from Hamburg to Santo Domingo

Table 1 Background concentration of atmospheric halocarbons							
Site of measurements	FCCs Chlorocarbons						
Western Ireland June/July 1974 North Atlantic October 1973 Chlorine equivalent	CCl <sub>2</sub> F <sub>2</sub> 101.7 (27.3) 115.2 (33.1) 470	CCl <sub>3</sub> F 79.8 (4.9) 88.6 (4.05)	CHCl <sub>3</sub> 26.5 (7.7) 18.9 (14.7)	CH <sub>3</sub> CCl <sub>3</sub> 64.8 (17.2) 75.1 (9.2)	CCl <sub>4</sub> 110.9 (10.7) 137.6 (14.7) 895	CHCl = CCl <sub>2</sub> 15.0 (12.1) <5 (5.7)	$CCl_2 = CCl_2$ 27.6 (9.3) 21.3 (3.25)

Concentrations including Cl equivalent all in parts per  $10^{12}$  by volume, figures within brackets are standard deviations. Note:  $CCl_2 = CCl_2$  has been characterised by retention time coincidence only.

in October 1973. Figure 1 illustrates the profile of concentration of CCl<sub>3</sub>F and CCl<sub>4</sub> in the troposphere and lower stratosphere over the United Kingdom in June 1974.

The chlorocarbons are less chemically stable than the FCCs but this seems not to hinder their transport to the stratosphere. If we assume that the abundancies listed in Table 1 are representative of the troposphere generally then the proportion of the total chlorine transferred to the stratosphere by each compound will be in proportion to their mixing ratios and chlorine contents. These proportions are listed for each compound in the third row of Table 1 and suggest a Cl concentration at the tropopause of at least  $1.4 \times 10^{-9}$ . This is more than ten times larger than the estimate of inorganic Cl for this region<sup>8,9</sup>.

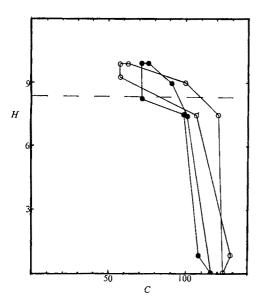


Fig. 1 Concentration (C) in parts per 10<sup>12</sup> by volume of CCl<sub>3</sub>F (●) and of CCl<sub>4</sub> (○) over central England on June 6, 1974 at heights (H) in km. - - -, Height of tropopause.

The FCCs seemingly contribute 35% of the total halocarbon chlorine entering the stratosphere. It is probable that this estimate is too high, since Table 1 lists only those compounds which have been observed. It is a commonplace for an unjustifiable significance to be attached to the easily measured quantity. It happens that the analytical method used is highly sensitive for CCl<sub>3</sub>F and CCl<sub>4</sub>. By contrast CH<sub>2</sub>Cl<sub>2</sub> and CH<sub>3</sub>Cl have not yet been sought because they are more difficult to detect. It would be sensible to determine the total organic chlorine content of the troposphere. It seems also probable that other chlorine compounds are there.

At first glance there would seem to be nothing unusual about the presence of traces of CCl<sub>4</sub> in the air. It is a typical organochlorine pollutant, a product of chemical industry. Closer investigation reveals that the origins of CCl<sub>4</sub> are tantalisingly obscure. R. L. McCarthy (personal communication) estimates that the sum total of  $CCl_4$  released to the atmosphere by all manufacturers and users of this compound does not exceed  $10^5$  ton up to the end of 1972. This quantity, had it accumulated without loss, would have given an atmospheric concentration of  $2.5 \times 10^{-12}$ . This is forty times less than the present abundance—moreover,  $CCl_4$  does not accumulate without loss; the soil, the sea and the stratosphere are all sinks<sup>5,10</sup>. There may be some unlisted or incidental production and release of  $CCl_4$  in industrial processes where chlorine encounters carbonaceous material. It seems unlikely that these unintentional sources are large; if they were it would surely be recognised, if only as a serious health hazard.

What then is the source of CCl<sub>4</sub>? Marine algae emit halocarbons<sup>5</sup> and a biological source of chlorocarbons is possible but its strength is unknown. A natural, probably atmospheric, origin of CCl<sub>4</sub> must also be considered as follows:

- The abundance of CCl<sub>4</sub> does not differ appreciably between the Northern and Southern Hemispheres<sup>5,6</sup>. This is not consistent with a northern industrial source.
- Air arriving at western Ireland shows a strong correlation between the concentration of the FCCs and Continental European origin. No such correlation is observed with CCl<sub>4</sub>.
- 3) Preliminary laboratory experiments in glass vessels show that the reaction in air between CH<sub>4</sub> and Cl<sub>2</sub> each at a concentration of 10<sup>-4</sup> results in the production of small but significant quantities of CCl<sub>4</sub>.

This evidence suggests a natural origin in the atmosphere itself. Chemical kinetics does not favour the direct gas phase chlorination of methane in the atmosphere but a heterogeneous atmospheric source is possible. If this is confirmed then other, as yet undiscovered, intermediates such as CH<sub>3</sub>Cl and CH<sub>2</sub>Cl<sub>2</sub> are likely also to be present. There is some indication from their spatial distribution that CHCl<sub>3</sub> and CH<sub>3</sub>CCl<sub>3</sub> may also in part at least have a similar natural origin to CCl<sub>4</sub>.

Molina and Rowland rightly warn of a potential hazard to stratospheric ozone should the emissions of the FCCs continue to grow unchecked but the prompt release of chlorine in the stratosphere from the other, non-FCC, halocarbons implies that the present input of odd chlorine species to the stratosphere is dominated by the simple chlorocarbons CCl<sub>4</sub>, CHCl<sub>3</sub> and CH<sub>3</sub>CCl<sub>3</sub> and so on. Indeed this input applied to the calculations of Wofsy and McElroy<sup>1</sup> indicates a 2% reduction of stratospheric ozone attributable to their presence. Unless there is some special additional effect from the release of chlorine at higher altitudes there are no grounds for singling out the FCCs as more hazardous than the other halocarbons which penetrate the tropopause.

In our paper on the distribution of halocarbons over the Atlantic<sup>5</sup> we unwisely commented: "The presence of these compounds (the FCCs) constitutes no conceivable hazard". It may be as unwise to assume that we are now at the early stages of a serious global pollution incident. We need urgently to discover the sources and sinks of CCl<sub>4</sub> and similar chlorocarbons, and also the stratospheric sinks for odd chlorine. If there is a sizeable natural chlorocarbon cycle it will give, so to speak, a stay of execution.

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#### Accuracy of weather forecasts

AFTER nine years of publication there has been considerable criticism of the usefulness and accuracy of long range weather forecasts. Gordon¹ has suggested that the forecasts have not yet attained a practical or economical degree of usefulness, and that advance computer technology and statistical power will not improve their accuracy unless a breakthrough in ideas is achieved. Existing methods are broadly based on an analogue or matching technique in which overall weather circulations of a particular month for many decades in the past are compared with those of the month preceding that for which the forecast is to be made.

It is, therefore, clearly necessary that various assessments of the results are made independently of those calculated by the predictors.

I have devised a classification to evaluate the accuracy of the predictions for the London area, from observations made at Kew Observatory. The portion of the long range forecast used is that part which refers to southern or south-eastern England. Although the forecasts mention several parameters, I have selected temperature and rainfall for verification (probably the two most important factors for the consumer, whether in the public or private sector of the national economy). This study deals with the 108 months from June 1965 to May 1974, inclusive.

Table 1 Assessment of official long range forecasts Predicted Good Bad Very bad Actual distribution Observed Above Temperature average 10 Ráinfall 5 31 32 22 Average Temperature Rainfall 36 38 Below 44 39 average Temperature 16 13 6 11 Rainfall

	Tal	ble 2 Scoring in systems A and B	
	forecast above below average		All forecast average
	$\begin{array}{c} 2 \times 3 = 6 \\ 1 \times -2 = -2 \end{array}$	(assuming the expected distri-	$ 2 \times -2 = -4 \\ 1 \times 8 = 8 $
A	$2 \times -2 = -4$ Total 0	bution of 2, 1, 2 in 5 trials	$2\times -2 = -4$
В	$2 \times 4 = 8$ $1 \times -2 = -2$ $2 \times -3 = -6$ Total 0		$2 \times -2 = -4$ $1 \times 8 = 8$ $2 \times -2 = -4$ $0$

The forecasts predict mainly one of three probabilities, below average, average (which includes near average or about average), and above average. A few forecasts of temperature have predicted much above average or much below average but these classifications have been treated simply as above average or below average in this study.

The boundaries which separate the classes above average, average, and below average are not published with the forecasts in the press or in the Weather Log (in Weather) and in many cases the public must make an inspired personal judgment. The Meteorological Office will, however, supply this information on request and it is understood that subscribers to the long range forecasts are provided with the exact boundaries. These are quintiles for temperature and terciles for rainfall, and are based on the climatic mean for the particular month and area of the United Kingdom for which the forecast applies. In the case of rainfall,  $\pm 20\%$  of the mean climatic total defines the

	Table 3 Sequential annual point totals 1965-74, inclusive (108 cases)						
Year		Temper	rature	Rain	nfall		
		, <b>A</b>	В	Α	В		
1965* 1966 1967 1968 1969 1970 1971 1972 1973 1974† Total		1 16 -4 -9 6 21 -14 -9 -9	3 19 -2 -10 8 27 -17 -11 -10 0	-1 3 -3 -3 3 0 3 -2	-1 2 -4 -4 5 1 0 5 -5 4		

- \* 7 months.
- † 5 months.

middle tercile or band width for the average condition. In the case of temperature the middle quintile for Kew is about  $\pm 0.35^{\circ}$  C when averaged throughout the year; it is used in the forecast assessment to define the average condition.

The classification system used here to assess the forecast results considers nine boxes, three for each class of prediction. If the observed result falls in the same box as the predicted result the forecast is 'good', if in a box adjacent to the predicted result the forecast is 'bad', and if in a box two boxes away from the predicted result the forecast is 'very bad'. In the last case it means that a forecast for above or below average conditions has been made whereas the observed result has been the opposite, that is, below or above average, respectively.

Table 1 shows the results for temperature and rainfall, together with the actual distribution of observed results. The observed quintile distribution follows closely the ratios 2,1,2 where much above and much below average are included with the above and below average cases. The observed tercile distribution follows the 1,1,1 ratios acceptably well for the rainfall.

Two scoring systems may be used. The first is that applicable to a game of chance. The second is rather better for the purpose of testing a scientific prediction technique in that it gives a one point bonus for a correct above average or below average forecast and a one point penalty for a very bad forecast as already defined.

For temperature, the quintile distribution is divided into the proportions 2,1,2. The probabilities for each class above average, average and below average, respectively, are therefore 2/5, 1/5, and 2/5. These three probabilities are equivalent to odds of 1.5 to 1, 4 to 1, and 1.5 to 1, respectively (3 to 2, 8 to 2, and 3 to 2, respectively). The gains for a success are therefore 3 for above average, 8 for an average and 3 for a below average. A failure in any class loses 2 points and this is indicated by -2. That explains the scoring system if the rules of a game of chance are operated (system A).

Scoring system B is exactly the same as system A except for a modification which allows arbitrarily one point extra if a success is forecast for above average or below average. Thus, a success for above average is 4, for average 8 as before, and for below average 4. To equalise this a penalty of one point is invoked if the result is the opposite of the forecast of condition. For the latter case the loss is -3 instead of -2.

Both systems add up to zero if all cases are forecast in any one category (Table 2). The total scores for the nine-year period studied are:

Temperature: system A, -1; system B, 7. Rainfall: system A, 6; system B, 4.

None of the scores approaches an acceptable level of statistical significance as given by the chi square test for 108 cases. Wright and Flood<sup>2</sup> found no statistical significance for the temperature and rainfall forecasts in an assessment covering the results of forecasts for the whole country for a two-year period. Some statistical significance was, however, found for the general information forecasts. The latter comprise statements of the kind "soon becoming wetter", "dry weather at first", "some warm days", and so on. In view of the fact that such statements are often very subjective in character it is extremely difficult to assess the results quantitatively. For this reason, and because the predictions are often of a synoptic scale type and, therefore, not really long range forecasts, it is doubtful if the significance is meaningful in the context of a 30-d period.

The results of the long range forecasts for a month ahead for temperature and rainfall do not approach an acceptable level of statistical significance. In fact, it is doubtful whether one can honestly say that they are even marginally better than chance. Although the selected cases only represent a sample of the total population of cases which could include a further 10 areas, the London area could be considered as one of the most important for verification. Further studies on the other areas should be done but it is doubtful if the net result would show any marked improvement. Furthermore, it is extremely doubtful that Ratcliffe's claim³ of a modest but real improvement in the last few years in the long range forecasts is now valid. Table 3 shows the year-by-year scores for systems A and B for both temperature and rainfall.

It is also questionable whether the band width of  $\pm 0.35^{\circ}$  C for the middle quintile for temperature is at all meaningful as a definition of average temperature for the consumer. A band width of  $\pm 1^{\circ}$  C might be far more useful to the suppliers of gas, electricity, coal and so on, for planning purposes.

It is certainly time to consider whether the very considerable expense incurred by this branch of our National Meteorological Service should continue to support what might be judged as a profitless activity. Bearing in mind that it is recognised that there is a built-in persistence factor in the sequence of

meteorological conditions, almost any person conscious of the weather should be able to obtain a small positive score after a sufficient number of trials, given the previous months anomalies.

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### Microstress mechanism for the time dependence of the modulus of crystalline polymers following an imposed change in volume

Parry and Tabor¹ have observed a significant time dependence of the dynamic shear modulus (G') of crystalline polymers at high hydrostatic pressure  $(10,000 \text{ pound inch}^{-2})$ . The modulus observed a few seconds after the increase in pressure is lower than the modulus observed at later times. There are two obvious explanations of this effect. The first is that it is due to a time-dependent bulk compliance (B). A pressure-induced reduction in volume increases G'. If B is time dependent, then the volume will also be time dependent and will continue to decrease after the increase in pressure, thus rendering G' time dependent. The second explanation is that the time dependence of G' is due to the pressure-induced generation of shear stresses in the heterogeneous solid. Of these explanations Parry and Tabor¹ favour the second. This view is supported by the following argument.

It is known that in polycrystalline solids with anisotropic thermal expansion coefficients, a change in temperature produces profound changes in both the plastic properties of the solid and the creep compliance<sup>2-6</sup>. This effect is attributed to the thermally induced generation of microstresses. The volume change of each anisotropic unit is constrained by the aggregate so that shear stresses are induced. The shear stresses relax slowly with time as the units shear. If the solid is stressed at the time of the temperature change the effective stress is the sum of the applied stress tensor and the microstress tensor and it is this combination of stresses, together with the nonlinear properties of the solid, which produces the anomalous mechanical properties.

The preceding mechanism is independent of the sign of the imposed change in volume. Evidence that this is in accord with observation is shown in Fig. 1. A specimen of linear polyethylene (Hifax 1900) was heated to 50° C and maintained at that temperature for 12 h. It was then cooled slowly to 40° C. After 10 min the temperature was increased abruptly (within 1 s) by  $\Delta T = +2.8^{\circ}$  C. A series of stress pulses (of duration 100 s) was then applied at various times after the jump in temperature. The interval between the stress pulses was sufficiently long that the strain had recovered to zero before the next stress pulse was applied. A second experiment was then performed, identical to the first in every way except that the temperature was reduced abruptly by  $\Delta T = -2.8^{\circ}$  C. Fig. 1 shows the time dependence of the observed shear strains at 40 s. 1,000 s and 6 h after the abrupt change in temperature. For both positive and negative values of  $\Delta T$  the creep strains decrease with storage time. That is to say, although the thermally induced

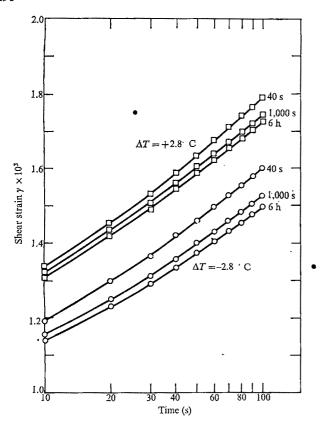


Fig. 1 Dependence of shear strain on time in successive 100-s pulse experiments for linear polyethylene showing the systematic reduction in strain with storage time for both positive and negative values of  $\Delta T$ . Temperature cycle:  $\uparrow$  50° C; 10 min;  $\Delta T$ ; pulse experiments. Shear stress  $\sigma_0 = 0.175 \text{ N mm}^{-2}$ .

volume changes were positive and negative the effect of storage time is always to increase the modulus.

The preceding experiment disposes of alternative mechanisms, such as time dependence of volume. It also leads us to predict that if the interpretation of Parry and Tabor<sup>1</sup> is correct then the observed value of G' in their experiments will always increase with storage time after a pressure change, independent of the sign of  $\Delta p$ . The crucial experiment will be to determine the time dependence of G' following a negative  $\Delta p$ .

It will be noted that the effect of lapse time is more marked in Fig. 1 for  $-\Delta T$  than for  $+\Delta T$ . This effect is due to the thermal history: the specimen was cooled to 40° C from 50° C. If the thermal cycle is altered in this respect alone (†50°C;  $\downarrow$  30° C; 12 h;  $\uparrow$ 40° C; 10 min;  $\Delta T$ ; pulse experiment) then the effect of lapse time is more marked for  $+\Delta T$  than for  $-\Delta T$ . The explanation is that the specimen is to some extent equilibrated for either  $+\Delta T$  or  $-\Delta T$  depending on the direction of approach to 40° C before the jump in temperature.

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### Effects of non-electrolytes on the solubilities of salts in water

It was shown by Setchenow<sup>1,2</sup> that the solubility  $(s_w)$  of a non-electrolyte in an aqueous solution of salt concentration $c_s$  was given by

$$\log s_{\rm w} = \log s_0 + k_{\rm s} c_{\rm s} \tag{1}$$

where  $s_0$  is the solubility of the non-electrolyte in pure water and  $k_s$  is a constant. Deno and Spink<sup>3</sup> found that for a given salt,  $k_s$  was proportional to the characteristic volume of the non-electrolyte which in m3 mol-1 equals the parachor (calculated in the usual way4 in c.g.s. units)×10-6. The effects on the solubilities of salts in water of the addition of many nonelectrolytes is now shown to depend also on the characteristic volumes as well as on the concentrations of these non-electro-

Rothmund<sup>5</sup> found that equation (2) could be used for the solubilities of lithium carbonate in water containing non-•electrolytes where  $S_{w}$  and  $S_{0}$  are the solubilities of the salt in the solution of non-electrolyte (concentration  $C_s$ ) and in pure water, respectively. Rothmund also measured the solubilities of silver sulphate, potassium bromate, potassium perchlorate and hydrated strontium hydroxide in water and in solutions (500 mol m<sup>-3</sup>) of non-electrolytes and found that, in general, the order for the effect of non-electrolytes was the same for each salt. It has now been found that K<sub>s</sub> is proportional to the characteristic volumes  $V^*$  of the non-electrolytes and that equation (3) can be applied to the solubilities of salts in aqueous solutions of many non-electrolytes.

$$\log S_{w} = \log S_{0} + K_{s}C_{s} \tag{2}$$

$$\log S_0$$
 (a constant for a given salt)= $\log S_w + k'_s V^* C_s$  (3)

Weber<sup>6</sup> carried out experiments similar to those of Rothmund. In Table 1 the results of Weber for potassium sulphate at 298.16 K have been used and, for the non-electrolytes listed, results for lithium carbonate<sup>5,6</sup>, potassium bromate<sup>5</sup>, silver sulphate<sup>5</sup>, silver bromate<sup>7</sup> and lead sulphate<sup>8-10</sup> at 298.16 K are given. The values of  $k'_s$  used were derived from all results reported and not just from those listed in Table 1. As required. by equation (3) values of  $\log_{10} S_w + k'_s V^* C_s$  are indeed, in most cases equal to  $\log_{10}S_0$  for a given salt. Some of the solutions of non-electrolyte are quite concentrated. For equation (3) to apply, the salt should be practically insoluble in the pure non-electrolyte which, of course, should not react chemically with the salt. Rothmund<sup>5</sup> showed that silver ions complex with ammonia and with amines. From Table 1, it would seem that silver ions also combine with acetonitrile and with phenol.

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Table 1	Effects	of non-el	lectrolytes	on so	lubilities	of salts	in w	ater at	298.16 K

Non-electrolyte and its characteristic			Formula of salt (v	value of $k'_{\rm s}$ )		
volume (V*, m³ mol-1)	$KBrO_3$ (0.8)	AgBrO <sub>3</sub> (0.6)	K <sub>2</sub> SO <sub>4</sub> (1.0) mol m <sup>-3</sup> ) and corre	Ag <sub>2</sub> SO <sub>4</sub> (1.1) sponding value of lo	$\text{Li}_2\text{CO}_3$ (1.05)	PbSO <sub>4</sub> (1.6)
None	2.679	0.910	2.745	1.427	${2.227 \brace 2.228}$	$\left\{ egin{array}{l} -0.824 \\ -0.829 \end{array} \right\}$
Methanol (8.74×10 <sup>-5</sup> )	500 2.682	3,064 0.902 6,021 0.895 8,890 0.889 11,620 0.867 14,240 0.840 16,680 0.794	125 2.745 250 2.746 500 2.743 1,000 2.742	500 1.444	250 2.228 500 2.230 1,000 2.236	,
Ethanol (1.2735×10 <sup>-4</sup> )	500 2.675	2,128 0.905 4,198 0.908 6,188 0.917 8,090 0.913 9,880 0.889	125 2.745 250 2.747 500 2.743 1,000 2.732	500 1.428	125 2.225 250 2.225 500 2.218 1,000 2.214	811 -0.830 1,732 -0.864 2,128 -0.834 4,198 -0.823 6,188 -0.858 8,090 -0.953 9,880 -0.782
<i>n</i> -Propanol (1.673×10 <sup>-4</sup> )	500 2.679	1,636 0.927 3,220 0.963 4,730 0.995 6,170 1.001 7,540 0.966	125 2.746 250 2.747 500 2.744 1,000 2.754	500 1.431	125 2.227 250 2.227 500 2.228 1,000 2.216	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,
t-Amyl alcohol (2.472×10 <sup>-4</sup> )	500 2.682		125 2.745 250 2.743 500 2.741 1,000 2.746	500 1.444	125 2.227 250 2.224 500 2.218 1,000 2.213	
i-Amyl alcohol (2.472×10-4) Acetone (1.551×10-4)	500 2.690	1,696 0.930 3,343 0.944 4,925 0.935 6,440 0.907	125 2.743 125 2.745 250 2.745 500 2.738 1,000 2.738	500 1.428	62.5 2.226 125 2.225 125 2.225 250 2.221 500 2.222 1,000 2.206	699 —0.862 1,413 —0.948
Butan-2-one (1.9505×10 <sup>-4</sup> )		3,110 0.50	125 2.749 250 2.750 500 2.755 1,000 2.769	•	2,000 <b>2.20</b> 0	
Diethylamine (2.189×10 <sup>-4</sup> )	500 2.672		125 2.751 250 2.757 500 2.774 1,000 2.783		125 2.230 250 2.228 500 2.223 1,000 2.202	
Acetonitrile (1.146×10 <sup>-4</sup> )			125 2.747 250 2.750 1,000 2.751	500 1.783	125 2.224 250 2.222 500 2.215 1,000 2.191	
Paraldehyde (2.8975×10 <sup>-4</sup> )			125 2.755 250 2.765 500 2.777		62.5 2.231 125 2.235 250 2.242	
Phenol (2.1975×10 <sup>-4</sup> )	500 2.717		125 2.751 250 2.757 500 2.769	500 1.700		
Methyl acetate $(1.7175 \times 10^{-4})$	500 2.692		125 2.755 250 2.763 500 2.779 1,000 2.809	500 1.421		
Ethyl acetate (2.117×10 <sup>-4</sup> )			125 2.756 250 2.768 500 2.790			ø

### Maternal nutrition and the sex ratio at birth

Many experiments on laboratory animals have shown that the nutrition of the dam can affect both litter size and viability. An early study indicated that breeding pairs of rats fed a poor diet also had an altered sex ratio in their offspring<sup>1</sup>, but no more precise nutritional studies of this have been made. Nutritional factors have not been considered amongst the determinants of the sex ratio at birth.

Female albino mice (Tuck and Sons, Essex) aged 35 d were

divided into two weight-matched groups and fed semipurified diets: either a low fat (LF) diet or a control diet. The diets differed only in their lipid composition. The control diet contained 66 g kg<sup>-1</sup> lipid, the low-fat diet 6 g kg<sup>-1</sup>. In the control diet, 5.0% of the dietary energy was derived from linoleic acid, 1.0% from linolenic acid. In the low-fat diet 0.27% of the dietary energy was as linoleic, 0.06% as linolenic acid<sup>2</sup>.

The control diet has been shown in this laboratory to support rats and mice through three generations, the low-fat diet to

induce essential fatty acid (EFA) deficiency in rats and mice. Animals were housed at 27° C (range 26-28° C) at a relative humidity of 80% (range 70-90%).

After 12 weeks on the diet, the animals were mated with males of the same strain that had been fed a standard laboratory diet (Diet 86, Dixons Ltd). Two females were caged with each male for 7 d during which time the males received the same diet as the females. Each male was used for only one mating.

Pregnant dams were caged individually 20 d after the introduction of the male and cages examined at least once a day for the presence of litters. The experiment was repeated three

The results presented in Table 1 show that still births and cannibalism were increased and litter size reduced on the low-fat diet, a result consistent with previous reports<sup>2,3</sup>.

What has not been previously demonstrated, however, is that the marked reduction in litter size is attributable almost completely to a reduction in the number of male pups born, no significant change occurring in the number of female births. The deviation in sex ratio at birth was due to this deficit in males relative to the control group and not to any excess in females. In spite of variations in litter size, in no litter born to a female on the LF diet was there an excess of male over female births. The unlikely possibility of parthenogenesis has been suggested when such sex ratios have been observed in mice, but this has found no experimental support<sup>4</sup>. Thus, either the Y sperm were less efficacious, or male foetuses were less viable in utero. The former possibility is unlikely, as EFA deficiency lesions take a considerable time to appear in animals fed a low-fat diet<sup>5</sup> and the male mice only ate the LF diet for 7 d during mating.

Table 1 The effect of diet on the number of male and female offspring

	Mate LF	rnal diet Control		
	diet	diet	Difference	P
No. litters born	26	28	•	
No. litters stillborn*	6	1	•	P < 0.05
No. litters partly or totally cannibalised No. litters examined	12	2	*•	P < 0.01
for sex ratio	8	15†		
No. pups per litter	5.13	7.94	2.81	< 0.025
No. male pups per litter	1.25	4.00	2.75	< 0.001
No. female pups per litter	3.88	3.94	0.06	n.s.
Sex ratio (males per 100 females)	32.2	101.5	69.3	< 0.05

Significance testing by  $\chi^2$  or Student's t test as appropriate.

Dead at first examination.

†10 litters excluded because no usable litters born to LF females from that mating.

Adult male mice have a much higher EFA requirement than females. The difference is presumed to be related to the known effects of sex hormones on EFA metabolism<sup>6</sup>. Some data suggest that the sex differences are not evident prior to sexual maturity7, although this point has been contested. The results of our experiment suggest that a sex difference in the sensitivity to EFA deficiency is not only established before sexual maturity, but before birth.

Marked distortions in sex ratio in some strains of mice are known—in one study on an inbred strain a sex ratio of 62 males per 100 females was obtained, but the cause of this remains 'still obscure'4. We suggest that the possibility of an elevated EFA requirement in such strains should be investigated. Our results indicate that even in common laboratory mice, foetal deaths can apparently be related to nutrient requirement. Therefore, it is possible that those male foetuses which survived had a lower EFA requirement or were more favourably positioned in utero. We are investigating this possibility and meanwhile suggest that the selection of progeny by nutrient requirement could complicate the interpretation of multigenerational experiments involving EFA deprivation<sup>2,3</sup>.

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#### Two modes of evolution

In some ways mammals evolve much faster than most other animals. In other ways however it now seems that they evolve at the standard rate. This dichotomy is quite unexpected, but seems post facto to be predicted by a new and ecological explanation of evolution.

Table 1 lists the phenomena whose relative rates are known. Faster turnover rates (origination and extinction) for mammals are well documented1,2 and can be shown in several ways not to be a taxonomic artifact (Van Valen, unpublished). Structural innovation and complexity is difficult to quantify, but it is commonly believed<sup>1</sup> to evolve unusually rapidly in mammals. The relative rates for chromosome number and hybrid inviability are from a comparison of frogs and mammals3.

The similar rate of amino acid substitution in proteins4.5 in mammals and other organisms is a well known problem. Its supplementation here with size evolution makes the problem worse. Kurtén<sup>6</sup> found that for ordinarily studied intervals of mammalian evolution (his "Tertiary" distribution), rates of measurable characters have a central tendency of about 30 millidarwins (ref. 7). There are almost no estimates of such rates for fish, amphibians, invertebrates, protists or plants. Because growth and evolution occur largely on a proportional rather than an absolute scale, logarithmic rates are appropriate1.7.

Table 1 Two modes of evolution in mammals with their associated phenomena

associated phonomera					
Mode	Phenomenon				
Standard	Protein sequences Linear measurements (size)				
Epistandard	Origination and extinction Structural innovation and complexity Chromosome number Hybrid inviability				

For another purpose I calculated 13 rates for invertebrates from data of Newell<sup>8</sup>. Because he may have selected his examples for higher rates, I then took the first 15 suitable references from my card file of evolutionary series and calculated rates from their data. Time scales are mostly from the Geological Society of London<sup>9</sup>, Kauffman<sup>10</sup>, or the original authors. Table 2 shows the results; the rates from Newell's data are comparable to the rest.

Although there are some patterns in the rates of Table 2, as a rough estimate of the central tendency we can use the logarithmic (geometric) mean of all the 75 rates weighted

Table 2 Evolutionary rates for invertebrates and protists, based on measurements and meristic characters (counts)

measurements	and meristic characters	(counts)		
Taxon	Character	Time Interval Rate (millions (milli- of years) darwins)		
Foraminifera		•	•	
Bolivinoides <sup>11</sup>	width	18	20,30	
Ataxophragmiidae <sup>11</sup>	' length	10	80	
Frondicularia <sup>11</sup>	width	2	300	
Gavelinellidae <sup>12</sup>	diameter	20	20	
Lagena <sup>13</sup>	length	6	140	
Orbitoides <sup>14</sup>	early diameter number early	6,7	60,100	
Clab 15	chambers	6,7	90,120	
Globoconusa15	proloculus diameter	4	40	
	final diameter	4	130	
Everlinidaes	number chambers	4 .	70	
Fusulinidae <sup>8</sup>	diameter	45	80	
Nummulitidae <sup>8</sup>	diameter	25	200	
Bryozoa Fenestrellinidae <sup>8</sup> Brachiopoda	aperture width	25	20	
Eocoelia <sup>16</sup>	width	5	90	
Locoena	number ribs	5	90	
Dictyothyris17	length	2	90	
Cyrtospirifer18	number ribs	5 8,15	100,0	
Gypidula <sup>19</sup>	beak height	2	140	
Terebratuloidea <sup>8</sup>	diameter	40	40	
Pentameroidea <sup>8</sup>	diameter	20	50	
Productacea <sup>8</sup>	diameter	20	140	
Richthofeniidae <sup>8</sup>	depth	40	40	
Rafinesquina <sup>8</sup>	width	20	50	
Punctospirifer8	diameter	30	50	
Pelecypoda	diameter	30	50	
Gryphaea <sup>20</sup>	width attachment	8	130	
Gryphaea	width shell	8	160	
Argopecten <sup>21</sup>	height	10,8,8, 7,2,	140,60, 30,10,	
	number costae	8,8 10,8,8, 7,2,	90,120,50 20,6,8, 30,190,	
		8,8	10,20	
<i>Myalina</i> <sup>8</sup> Ammonoidea	hinge length	50	40	
Pinacoceratidae <sup>8</sup> Ostracoda	diameter	35	40	
Cytherella <sup>22</sup>	length	7	3	
Ovocytheridea <sup>22</sup>	length	3	4	
Mehesella <sup>22</sup>	length	15	30	
Leguminocythereis <sup>22</sup>	length	3	70	
Cythereis <sup>22</sup>	length	8	9	
Trachyleberis <sup>22</sup>	length	3	9	
Buntonia <sup>22</sup>	length	3,3,2,25	40,40,8, 20	
Stelleroidea <sup>8</sup>	maximum width	100,350	15,5	
Echinoidea			-	
Micraster <sup>23</sup>	length	18,18	8,20	
Holasteridae <sup>24</sup>	length	5	260	
Melechinoidea <sup>8</sup>	height width	5 20	50 60	
Graptoloidea				
Neocucullograptinae <sup>26</sup>	length sicula	5,5,1,2, 1,3	20,30, 190,40, 50,80	
	length first theca	5,5,1,2, 1,3	4,30,160, 140,140,	

Individual values are accurate only to about a factor of 2, but presumably are unbiased.

equally. This is about 40 millidarwins, ostensibly even a little higher than the value for mammals. (The variation in both distributions, however, is large enough for no biologically real difference to be apparent.) Two previous comparisons<sup>26,27</sup> of size rates between invertebrates and mammals both gave invertebrates the higher rate, although each used less than a half dozen rates and neither noted the resulting paradox. The highest known rates of size evolution are similar for mammals<sup>6</sup> and protists<sup>28</sup>, about 20 darwins in each case (excluding human introductions).

Some mammalian structures participate in both modes of evolution and so may focus the problem. Horse teeth are an example. Their measurements evolve in the standard mode<sup>1</sup> but their patterns of enamel, dentine, and cement evolve in the epistandard mode, as judged directly but subjectively, and indirectly by the rates<sup>1,2</sup> of origination and extinction of taxa that are defined in part by their tooth pattern.

The difference between the modes clearly is not that of neutral against adaptive evolution. Measurable aspects of horse teeth are of obvious selective importance; strong natural selection has in fact been measured on them<sup>29</sup>. The degree of constancy for size evolution is nevertheless indistinguishable now from that of protein evolution, which has some more or less predictable deviations from constancy<sup>30,31</sup> and which is measured on a much grosser scale than is size evolution. The Red queen's hypothesis<sup>2,31</sup> predicts such a degree of constancy, and I have suggested<sup>31</sup>, without evidence, that fish have really evolved as fast as tetrapods.

How then to explain the epistandard mode? In the resource space<sup>32</sup> the proportion of the resources used by one species changes over evolutionary time; usually, if one species gains resources, others lose. Presumably the boundaries in the resource space between species shift back and forth, rather than there being mostly unidirectional changes. The Red queen proposes that the changes occur at a constant average rate. The rate of extinction depends on the susceptibility of the species at risk: the greater the proportion of its resources it loses, the greater the risk of extinction.

For mammals, then, the spectral threshold<sup>33</sup> of ultimately regulatory resources needed to prevent extinction is presumably lower (loss of a lower average proportion of resources causes extinction) than in most organisms. Mammals then become extinct more often than most organisms with similar fluctuations in the partitioning of the resource space. Epistandard origination rates are needed to replace the extinctions; perhaps this just involves greater survival of incipient species, which could themselves help cause the extinctions. The apparently epistandard evolution of morphological pattern is clearly consistent with this situation, as is the epistandard reshuffling of the genome. The latter effects, however, may not be necessary causal adjuncts.

And why should mammals be so susceptible to extinction, or have such a high rate of formation or survival of incipient species? There are several possible explanations, related to mammalian attributes such as the high rate at which they use energy, their apparently sharply bounded but contiguous adaptive zones, the ease with which their genomes became reorganised, or their high-level feedback systems. Whatever the resolution of this point, it is clear that phenomena related to origination and extinction rates should participate in the epistandard mode.

Thus, for a group whose taxa have a low threshold for extinction, such as mammals, some phenomena will evolve in the epistandard mode, in some sort of causal relation to this low threshold. But other phenomena will be unaffected by the causal nexus of the threshold and will evolve in relation to the standard losses and gains in resources (realised fitness). Possibly some phenomena are intermediate, and other groups are expected to participate in epistandard mode to the extent that their extinction rates are above normal.

In the zero-sum game of the Red queen each species competes, directly or indirectly, with all others, and no species ever wins;

new adversaries replace the losers. This world is stark and shifting; wherever an equal shift causes greater harm, as for mammals, we have the possibility of explaining the additional mode of evolution that occurs there.

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### Honey bee communication

More than fifty years ago von Frisch demonstrated that foraging honey bees aroused the interest of potential recruits by means of a 'dance'. These experiments showed that the forager's body provides sufficient information about the odour of the food source to enable other bees to find that food in the field. In addition von Frisch demonstrated that foragers also leave odours at the food source which aid recruits in their searches<sup>1,2</sup>. Von Frisch later found that the dance also contains distance and direction information which the recruits seem able to use3,4.

Recently von Frisch's controls have been criticised and new experiments have suggested that olfactory information alone is sufficient to explain the phenomenon of honey bee recruitment<sup>5-10</sup>. These experiments, however, were also subject to criticism11,12 and have stimulated new work with improved controls for odour cues 13-16. These more recent results are still subject to the interpretation that the bees might have been using a 'locality odour' instead<sup>17</sup>. Since there is evidence that bees can use such cues<sup>17,18</sup>, interpretation of previous work is seriously complicated. The experiments reported here were designed to examine this question more rigorously.

Under some circumstances honey bees seem to interpret a bright light in the hive as the Sun and will orient their dances to it rather than to gravity. Since both dancers and dance attenders are reoriented, no misdirection occurs19. When the three ocelli are painted over, however, a honey bee becomes far less sensitive to light 20,21 and when a light of an appropriate brightness is used, foragers with painted ocelli will ignore the light and dance with respect to gravity; while untreated bees both dance and interpret dances as though the light were the Sun. If the bees use the distance and direction information in the dance, it should be possible for ocelli-painted foragers to send recruit bees to specific but incorrect locations.

Two groups of foragers were trained using Gary's technique 22. The first group served as a control to measure the degree of reorientation of the dances to the light. The ocelli of the foragers of the second group were covered with flat black enamel during training. A 650 W quartz lamp shielded by a heat-absorbing filter was used as the light source. The hive was constructed so that dances occurred only on one side of one frame. The distance of the light from the dance area was varied until the dances of the control foragers-whose ocelli were unpainted-were completely reoriented, while those of the ocelli-painted foragers were not. For 30 min before the experiments began, the light was left on to adapt the bees to its presence. It illuminated the dance area from above, that is, the direction away from gravity. Since that is the direction 'assigned' to the Sun when the dances are performed in the dark, the dance orientations were not affected. At the beginning of the experiment the light was moved to a different angle, and the solution at the ocellipainted forager station was changed to 2 M peppermint-scented

Six experimental stations similar to those designed by Renner<sup>23</sup>, and almost identical to the forager station, were set out at the positions shown in Fig. 1. Because many recruits

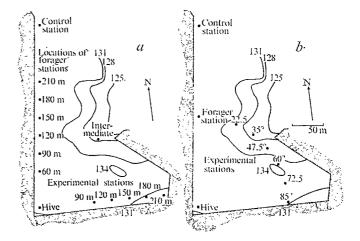


Fig. 1 Experimental array for distance and direction experiments. One group of foragers was trained on 1 M anise-scented sucrose to the control station 300 m to the north. A second group, whose ocelli were covered with paint, were trained in the same direction on 0.5 M orange-scented sucrose. Elevations are given in metres. a, Distance experiment, five experimental stations were placed in a line to the east at 30 m intervals. A sixth intermediate station was placed at the location shown, 150 m from the hive. A light in the hive caused recruits to misinterpret the dance of the treated foragers and to search for the food to the east rather than to the north. The forager station was moved every 20 min to a new location. This altered the distribution of recruits at the experimental stations (see Fig. 2). The wind was from 159° at 2 km h-1 b, Direction experiment, six experimental stations were set out at 12.5° intervals at 150 m from the hive. Every 30 min the light in the hive was moved, altering the interpretation of the dance direction. This altered the distribution of recruits (see Fig. 3). The wind was from 001° at 10 km h-1.

are hesitant to land unless they can see other bees already there, the 'flower'24 of each station was 'baited' visually with the body of an alcohol-extracted, Sun-dried bee. Each station was 'odour-baited' with the experimental solution and a wire box containing 10 anaesthetised, ocelli-treated foragers captured during training. Entering bees interrupted a beam of red light and the resulting signals from a photocell were recorded on a six-channel event recorder. The stations were filled with CO. which anaesthetised the recruited bees while they fed. Recruits arriving at and entering these stations therefore never returned to the hive to dance.

Recruits arriving at the ocelli-treated forager station were captured without the release of odours by a method described previously<sup>13</sup>. Wind speed and direction were automatically recorded while other weather parameters were monitored every 10 min. The dances of both control and ocelli-treated foragers in the hive were measured from video recordings, and the visits of treated foragers to their stations were recorded.

The first experiment was performed to determine whether information about distance is conveyed by the dances. The light was moved 85° to the left of vertical. As a result the dances of the foragers with painted ocelli were 85° to the right (clockwise) of those of the reoriented control foragers, and would therefore be expected to misdirect untreated bees to the right ' by 85°. The experimental array was located 85° to the right of the forager station (Fig. 1a). The forager station was moved every 20 min to a new distance in the following order; 90 m, 120 m, 150 m, 180 m, 210 m, 150 m, 90 m, and 60 m. Recruits arriving along the experimental array did show a preference for the distance indicated by the dance (Fig. 2).

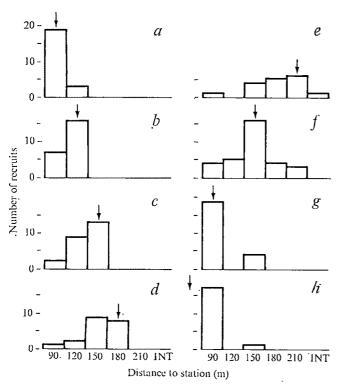


Fig. 2 Results of the distance experiment. The number of recruits captured at various distances from the hive along the experimental array is shown for each location of the forager station. The distance of the forager station is indicated by the arrow (in h the distance was 60 m). Each bar graph represents a 20 minute interval.

The second experiment was performed to determine whether directional information is communicated by the dance. The light was moved every 30 min, first 85° to the left of vertical, then to 35° left, and then to 60° left. The control foragers were reoriented by the same amount, so the recruits would be expected to misinterpret the dances of the treated foragers by a

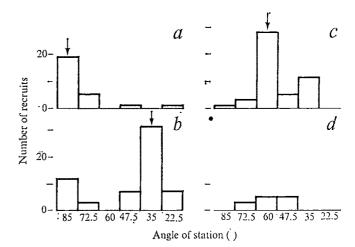


Fig. 3 Results of the direction experiment. The number of recruits captured in various directions from the hive is plotted for several orientations of the light. The arrow indicates the direction which the treated foragers signalled to untreated recruits. The timeintervalis 30 min. In d the light was moved to the 'up' position and the forager food removed.

corresponding angle to the right. The experimental array was composed of six stations arranged at 12.5° intervals and 150 m from the hive (Fig. 1b). The recruits arriving at the experimental stations did show a preference for the direction expected from the dance (Fig. 3).

Since most of the recruits arrived at locations specified by the dances, but not near the site being frequented by the foragers performing those dances, olfactory information unique to the forager station or its locale does not explain recruitment. Under these conditions the dance clearly communicates abstract distance and direction information which many recruits are subsequently able to use.

Additional details of this technique, these experiments and the results of subsequent experiments will be reported elsewhere. I thank Donald R. Griffin, Peter Marler and Fernando Nottebohm for their criticisms of this manuscript. Financial support was provided, in part, by the Cary Trust, Millbrook, NY.

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### Erythropoiesis in a cell population from the early chick blastodisc induced by diffusible product of second cell population

Onset of rapid haemoglobin formation in the blood islands of the developing chick blastodisc, both in ovo and in vitro, commences at the stage of the 6- to 7-somite embryo<sup>1</sup> which is normally attained after 28-33 h of incubation. This phase of haemoglobin formation seems4,6 to reflect a previous covert differentiation of erythroid-progenitor cells distinct from pre-existing erythropoietic cells which form small quantities of haemoglobin as early as the primitive streak stage of development—after about 20 h incubation<sup>5</sup>.

We have recently developed procedures for the isolation from dispersed single-cell suspensions of primitive streak and head-fold blastodiscs of two distinct cell populations which form foci of erythropoiesis readily visible to the naked eye when incubated as cell reaggregates. One of these populations was further resolved into two discrete essential subpopulations. Aggregates of either of the individual subpopulations tested singly formed no detectable haemoglobin, whereas aggregates formed by remixing portions of the resolved subpopulations were strongly erythropoietic<sup>6</sup>.

As a first approach to defining the nature of the interaction between these two subpopulations we have now determined that direct contact between them is not required. Rather, one of the subpopulations contains progenitors of cells of the erythropoietic series which differentiate into erythroid cells in response to diffusible product(s) of the complementary subpopulation.

Blastodiscs of the Shaver Starcross No. 288 line of White Leghorn fowl were explanted on to a solid minimal medium<sup>3</sup> and those at the primitive streak and head-fold stages of development were selected for these experiments. They were detached from their supporting vitelline membranes and dispersed by incubation with gentle agitation for a total of 20 min in 125 µl per blastodisc of minimal medium containing 40 units ml-1 of collagenase (Sigma Chemical Company, Type I) and 50 units ml<sup>-1</sup> of hyaluronidase (Sigma Chemical Company, Type I). Dispersion was facilitated by gentle flushing with a Pasteur pipette at 10 and 20 min of incubation. Suspensions of single cells were recovered by filtration through one layer each of Nitex No. 73 (Thompson and Co., Montreal) and Nitex No. 53 nylon monofilament cloth.

Volumes (1.2 ml) of suspension containing  $1.2 \times 10^7$  cells ml<sup>-1</sup> were layered on to freshly-prepared, sterile discontinuous density gradients of Ficoll in liquid minimal medium, at room temperature. These consisted of a 1.3 ml upper layer of solution of specific gravity 1.044, a 1.3 ml intermediate layer of specific gravity 1.049, and a 1 ml cushion of specific gravity 1.062. These gradients were immediately centrifuged in Spinco SW 50 rotors at 4,500 r.p.m. for 30 min without braking. Cells retained at the upper (subpopulation A) and lower (subpopulation B) interfaces, respectively, of the upper Ficoll band of the gradient were each transferred to 5 ml of minimal medium -2% foetal calf serum (Gibco, Grand Island, New York) and sedimented at 5,800g for 10 min.

The pellet of subpopulation A was plated with a sterile microspatula on a portion of vitelline membrane laid on the surface of egg homogenate medium, freshly prepared and prewarmed. A layer of dialysis membrane sterilised by autoclaving in distilled water, then equilibrated with Earle's minimal medium containing HEPES buffer (Gibco, Grand Island) and 20% foetal calf serum, was placed over this aggregate and extending beyond it for at least 2 cm on all sides. The pellet of subpopulation B was then plated on the dialysis membrane, and the whole was incubated at 38° C in a moist chamber.

At 4 d the upper aggregate of subpopulation B was

seen to be 'peppered' throughout with foci of erythropoiesis readily visible to the naked eye. By contrast, neither the lower aggregate of subpopulation A nor control aggregates of subpopulation B plated alone on dialysis membrane contained any visible haemoglobin.

This result clearly establishes that the two resolved cell populations play different roles in the differentiation of cells of the erythropoietic line. Moreover, they demonstrate that the progenitors present in subpopulation B differentiate to erythroid cells in response to diffusible material(s) of low molecular weight produced by cells in the complementary subpopulation A.

Miura and Wilt<sup>8</sup> have previously demonstrated that haemoglobin formation in cells of the mesodermal layer of the primitive chick blastodisc is dependent upon material from cells of the endoderm layer which can diffuse through a Millipore filter. It is therefore tempting to suppose that the essential cells of our subpopulations A and B are derived from the endoderm and mesoderm, respectively. The relationships between the resolved subpopulations and those inferred to be present in the intact blastodisc however remain to be established.

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### Preparation of human placental villous surface membrane

THERE is currently considerable interest in the mechanism of protection of the foetus against rejection by the mother. Various theories have been put forward to explain this phenomenon, including the existence of an immunologically inert barrier between mother and foetus2, and a reduction of immunological reactivity of the mother during pregnancy3. A further possibility is that a hormone such as progesterone binds to the external villous surface of the placenta and masks its antigenicity.

The surface membrane of human chorionic villi represents the effective foeto-maternal junction and therefore a preparation of this membrane would be of value in studying the immunochemistry of the foeto-maternal relationship. We have used a simple saline extraction technique to remove this material from placenta and have studied its composition by electron microscopy. It is likely to contain any antigenic material present as a somewhat similar technique (hypotonic solutions, 0.1-0.8%) was used by Davies4 in the preparation of membrane fragments rich in H-2 antigenic activity from cell surfaces of mouse spleen and thymus.

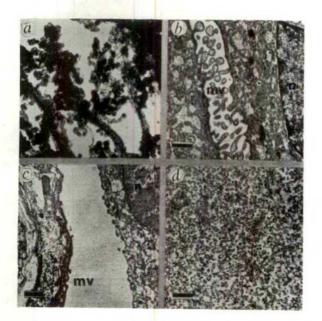
Freshly delivered normal human placentae, whose normality was subsequently confirmed by histology, were used. Preliminary experiments using solutions of varying composition and ionic strength established the optimal conditions. In the procedure finally adopted, a 10-20 g tissue sample is spread out manually and then washed rapidly, first in ice-cold isotonic CaCl2 solution and then in ice-cold Kreb's physiological saline. This removes much of the blood from the intervillous spaces. The sample is next placed in ice-cold 0.9% NaCl solution and gently agitated at 0° C for 30 min using a magnetic stirrer. During this procedure, the chorionic villi spread out so that their surfaces are well irrigated (Fig. 1a). The saline wash is then poured off and centrifuged for 10 min at 800g to remove fragments of tissue and red blood cells. The resulting cloudy supernatant is centrifuged in a Beckman Spinco at 10,000g for 5 min to remove larger particles and intracellular debris. Then a higher speed run (100,000g for 60 min) yields a pale jelly-like pellet.

For electron microscopie examination, three samples were taken: intact untreated placental tissue, tissue stirred with 0.9% NaCl solution for 30 min and the pellet obtained by centrifuging the NaCl wash at 100,000g. Each sample • was placed in 3% glutaraldehyde in buffered solution as

quickly as possible.

Examination of untreated tissue kept at 4° C for 40 min and compared with tissue fixed immediately (Fig. 1b), established that no significant deterioration takes place in that time but a striking loss of microvilli was seen in tissue washed with 0.9% NaCl solution (Fig. 1c). It was apparent on scanning several samples of the treated tissue, that many of the chorionic villous surfaces were free or almost free of microvilli and that no other significant changes had occurred, indicating that there had been little or no cellular damage except that due to removal of microvilli. The sedimented pellet (Fig. 1d) contained mainly membranebound bodies resembling microvilli. The material removed at 10,000g consisted of larger particles, probably consisting of intracellular fragments from damaged areas.

Preparations of the pellet of microvilli contained a high



a, Wet mount of chorionic villi floating in saline Fig. 1 a, Wet mount of chorionic vilil noating in saline  $(\times 60)$ ; b, Two adjacent villi before saline treatment; c, after saline treatment; d, 100,000g pellet. Samples for b, c and d were fixed in 2.5% glutaraldehyde in sodium cacodylate buffer, secondarily fixed with 1% osmic acid, dehydrated and then embedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate. Bars represent 1 μm in b, c and d. n, nucleus; mv, microvilli.

percentage of water (85-90%). Protein<sup>5</sup>, carbohydrate<sup>6</sup>, sialic acid<sup>7</sup> and lipid were also present. The progesterone content of the pellet was relatively high (2-5 µg per g wet weight which is significantly different (P=0.001) from that of whole placental tissue (1-2 µg per g wet weight)). Progesterone was estimated by gas-liquid chromatography after purification of the lipid extract by thin-layer chromatography on silica gel.

Work is now in progress on the nature of the association of progesterone with the villous surface of the placenta, as represented by the pellet of microvilli. After solubilisation by 1% sodium deoxycholate, the product is incubated with radioactive (1, 2-3H) progesterone and is then fractionated by ion exchange chromatography, gel filtration and

polyacrylamide gel electrophoresis.

We believe that the material removed from placenta by the treatment described consists of microvilli and related membrane fragments, and the evidence for this is the appearance of the detached particles and the fact that the tissue left behind is essentially normal under the electron microscope. The preliminary chemical examination of the

pellet is compatible with this view.

The procedure outlined above is simple, rapid and gives a good yield of material. Apart from further studies on the immunology of the foeto-maternal interface, it should also be a useful experimental preparation for general investigations on the biochemistry of cell surface antigens, membrane-bound enzymes and other membrane components. In view of the extremely marked degenerative changes in the trophoblast which are often seen in pre-eclampsia and sometimes in certain other disorders of pregnancy it may be useful to use this technique in the study of these conditions.

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### The effects of mitomycin C on remyelination in the peripheral nervous system

REMYELINATION within the central nervous system (CNS) is rarely seen in pathological material or in experimentally produced lesions and when it occurs it is a considerably slower process than its counterpart in the peripheral nervous system. It is generally thought that a major factor precluding central remyelination is the failure of oligodendroglia to undergo division1.2. As this hypothesis is difficult to test experimentally

in the CNS, we have attempted to mimic the situation peripherally, and have examined the effects of inhibiting Schwann cell proliferation after peripheral demyelination. Here we report our preliminary results.

This type of experiment is feasible only if the demyelination is not accompanied by destruction or injury of Schwann cell or axon, and a reasonably specific means of blocking cell division can be used in which side effects are minimal. We have shown previously that the intraneural injection of lysophosphatidyl choline (LPC) into the sciatic nerve of the adult mouse will produce a circumscribed demyelinating lesion, without producing detectable damage in either Schwann cell or axons<sup>3</sup>. Mitomycin C inhibits DNA replication<sup>4</sup> and has been used successfully to inhibit cell division in early frog embryos<sup>5</sup>.

In the first experimental series, adult mice received intraneural injections (about 2 × 10<sup>-4</sup> ml) of a solution of mitomycin C (400 µg<sup>-1</sup> ml in physiological saline), followed 24 h later by a solution of LPC (10 mg ml<sup>-1</sup> in physiological saline) (ML24 animals). Animals were killed at various intervals after the I•PC injections, and the nerves processed for conventional electron microscopy. Details of the method of injection are given elsewhere<sup>3</sup>. Controls received an intraneural injection of physiological saline followed by LPC (SL24 animals).

In a second series of mice, 1µ Ci ml<sup>-1</sup> <sup>3</sup>H-thymidine (specific activity about 5 Ci mmol<sup>-1</sup>) was injected intraneurally at intervals from 1 h to 48 d after the LPC injection; this was chased 45 min later by the intraneural injection of 12 mM thymidine. The animals were killed after 4 h. Controls received saline instead of LPC. A further group of ML24 mice received intraneural injections of <sup>3</sup>H-thymidine at various time intervals 3–20 d after the injection of LPC. Lengths of sciatic nerve were removed and either homogenised in cold 10% TCA, the residues collected on Millipore filters, washed and counted in liquid scintillation medium<sup>3</sup>, or they were stretched on card, processed as for electron microscopy, 3 µm thick resin sections cut and prepared for light microscope autoradiography.

In a third series of mice, standard intraneural injections of LPC were followed 3 or 7 d later by the intraneural injection of mitomycin C (LM3 and LM7 animals). The animals were killed 20 d after the LPC injections and the nerves processed for conventional electron microscopy.

There was an obvious behavioural difference between the ML24 and SL24 series. ML24 mice exhibited weakness of the hind limb which persisted for up to 14 week after the LPC injection; a similar weakness in the SL24 animals disappeared within the first 2 weeks.

Morphologically, the overall consequence of pretreatment with mitomycin C was a retardation of those changes which occur subsequent to LPC-induced demyelination. Thus, in ML24 mice the Schwann cells remained laden with debris for 2–3 weeks, while in the SL24 mice most of this debris was extruded from the axon-associated Schwann cells and taken up sequentially into supernumerary Schwann cells<sup>6,7</sup> and endoneurial macrophages within the first 10 d. There was a marked

4 h thymidine ur

10

Time after LPC

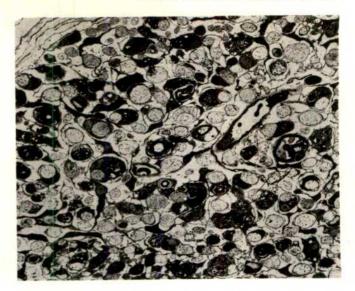


Fig. 1 ML24, 20 d after injection. There has been no remyelination (×980).

increase in Schwann cell number 6–10 d following demyelination in SL24 mice; there was little increase in Schwann cell number in those animals pretreated with mitomycin C, although in both groups there was an increase in the numbers of haematogenous cells and of fibroblasts. The relative uptake of <sup>3</sup>H-thymidine by SL24 and ML24 nerves indicated that most of this increase in cell number in SL24 mice was due to local cell division (table 1).

In SL24 mice, remyelination was established in at least 80% of the fibres by 20 d, with a concomitant maintenance of the high endoneurial nuclear count, reflecting the persistence of numerous axon-associated and supernumerary Schwann cells (about 15% of the axons were associated with Schwann cell nuclei)7. In ML24 mice, however, the Schwann cell nuclear count remained low during the first month after injection (4% of the axons were associated with Schwann cell nuclei, a figure within the limits for normal nerve). Transverse sections of ML24 nerve during this time revealed many axons to be incompletely surrounded by fine processes of Schwann cell cytoplasm, while others (about 25% of the total axonal population in some cases) appeared not to be associated with a Schwann cell. In all cases, the axons, with or without a Schwann cell, were enclosed by a continuous basal lamina. There was no evidence of remyelination (Fig. 1), although in a few Schwann cell/axon units in ML24 mice complex infoldings of Schwann cell plasma membrane were observed, unrelated to the enclosed axon, reminiscent of aberrant mesaxons in neonatal nerve. It is possible that these represented unsuccessful attempts at remyelination.

In ML24 mice many axons exhibited morphological features not normally observed in the axoplasm of normal or remyelinat-

uptake)

16

Table 1	Uptake of thymidine by LPC-demyelinated nerves with or without mitomycin C pretreatment					
iptake (c.p.m.						
C (d)	Controls (SL24) 2377 s.e.m. $\pm 449$ ( $n=3$ )	Mitomycin C-treated (ML24) 601 s.e.m. $\pm$ 172.8( $n$ = 6)	Test/Control (%			

140 s.e.m.  $\pm$  38.5(n=5)

10	052 S.C.III. T	293 (11-3)	140 5.0.111.	30.5(n-3)		1,000,000
4 h thymidine uptake,	differential cell count	SECTION SECTION				
Time after LPC (d)	Controls (LP	C)		Mitomycin C-treated (ML24)		
***	% labelled Schwann nuclei Axon-associa		iated Schwann % labelled Schwann nucle		Axon-associated Schwann nuclei	
		total	% labelled		total	% labelled
- 5	22+7.1 (n=3)		V. We Execution South	3.5 (n=2)		
6	$29 \pm 6.3 (n=3)$	129*	48*	2.0 (n=3)	_	_
10	6+5.3 (n=3)	356	9+8	6.0 (n=2)	-	_
18	$2 \qquad (n=1)$	64	3	_		_
20	$3 \qquad (n=2)$	187	4	7.0 (n=3)	_	_

<sup>\*</sup> In two samples, Schwann cells that were axon-associated were also debris-containing, so these data refer to one sample only.

832 s.e.m.  $\pm$  295 (n=3)

ing axons: large masses of myelin debris, often filling 80% of the axonal cross sectional area (Fig. 2), a proliferation of smooth endoplasmic reticulum, and numerous ribosomes. Intra-axonal lamellar debris was observed most commonly during the first 3 weeks after injection.

During the acute phase (1-20 d) of the response in ML24 mice, the morphology of the fibres 1 cm distal to the lesion was examined routinely in 1 µm resin sections, and found to be normal in 90% of the mice: however, in 10% some fibres appeared to be undergoing demyelination or Wallerian-type degeneration. In ML24 mice, 200 d after LPC injection, not only had remyelination occurred throughout the site of the lesion, but most fibres distally exhibited short thinly myelinated internodes indicating that they had also undergone demyelination and subsequent remyelination. It is possible that the proliferation of Schwann cells distal to the lesion is a compensatory response, provoked by chronic alterations in the Schwann cell/axon relationship.

In both LM3 and LM7 mice, the progress of the lesion was intermediate between that in the SL24 and ML24 animals. This is compatible with the hypothesis that it is the initial or early cell divisions which are critical in reactivating the myelinating activity of the Schwann cell.

Although in general these experiments support the idea that mitomycin C acts primarily by inhibiting DNA replication and thus cell division, its possible effects at the level of transcription must not be ignored. This aspect of mitomycin C action is being investigated.

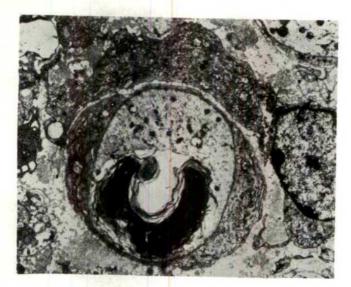


Fig. 2 ML24, 20 d after injection. Note the myelin debris in the axon  $A (\times 7,350)$ .

We conclude that the time course of the cellular response in the peripheral nervous system after the inhibition of satellite cell division at the time of a primary demyelinating episode closely resembles that found in the CNS after demyelination, that is, a slow removal of myelin debris and a failure of early remyelination. This suggests that the converse experiment, a stimulation of oligodendroglial division following primary demyelination in the CNS might lead to a more successful regenerative process

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### Phagocytosis by pigment epithelium of human retinal cones

AUTORADIOGRAPHIC studies of vertebrate retinae indicate that rod outer segment disks are continuously manufactured at the base of the outer segment1.2. The disks are slowly displaced towards the tip of the outer segment, where they are shed and phagocytosed in groups by the apical processes of pigment epithelial cells3.4. Identical autoradiographic studies indicate that cone disks are not continuously manufactured at the base of the outer segment, leading to the conclusion that they are not phagocytosed<sup>5</sup>. The observation that phagosomes occur within the pigment epithelial cells of the rod-free region of human foveola, however, suggested that outer segment disks from foveal cones are phagocytosed6. Are human foveal cones uniquely rod-like in this respect, just as they are rod-like in their form and in their extension to the pigment epithelial surface, or are the disks of all human cones phagocytosed by the pigment epithelium? Our observation of a close association between cone outer segments of cat retina and pigment epithelial cell processes7 suggested an answer. As with human extrafoveal cones, those in cat do not reach the pigment epithelial surface; instead, long leaf-like processes extend from the pigment epithelium to the outer segments. These cell processes form a multilaminar sheath around the external one-third of the outer segment. If these processes engage in phagocytosis, phagosomes containing groups of cone disks should be observed within their cytoplasm and in good sections there should be very little chance of confusing them with phagocytosed rod disks. In human retinae, where cones are more plentiful than in the cat, the extrafoveal cone outer segments are also closely associated with long pigment epithelial processes 8-11. We report here that these processes phagocytose groups of disks from the cone outer segements.

The retina used for this study was obtained from the left eye of a 5-yr-old patient with an orbital rhabdomyosarcoma. The eye did not show any pathological changes. Immediately after surgical removal, the eye was transected into anterior and posterior segments and placed in fixative. The posterior segment was then dissected to isolate the macular region (Fig. 1). Sections were cut (1 µm) for orientation purposes, stained with basic fuchsin and methylene blue and examined with a Zeiss photomicroscope. Ultrathin sections were cut from the aligned specimen, stained with uranyl acetate in H<sub>2</sub>O and lead citrate, and examined in a Siemens-Elmiskop 1A electron microscope.

Human extrafoveal cones are readily distinguished from the surrounding rods; cone outer segments are shorter than rod outer segments while cone inner segments are much wider than rod inner segments. Cones are also identified by long processes which reach their outer segments from the pigment epithelium. Some of these processes adjoin the cone outer segment for at least one-half of its length.

The basic features of the cone and its relation to the pigment epithelium are seen by light microscopy (Fig. 1). The pigment epithelial processes extend from the epithelial surface to occupy the space above the cone outer segment tips. Oval melanin granules occur within the processes close to the epithelial surface. There are also two irregular inclusions within the epithelial processes close to the outer segment of the cone on the left and there is at least one irregular inclusion in the processes of the cone on the right (arrows in Fig. 1). These inclusions stain like the outer segment but somewhat more intensely and may be phagosomes. Their identification as such can be made only with the electron microscope.

Of the cones surveyed by electron microscopy, at least onethird showed phagosomes within the epithelial processes. There is usually more than one phagosome within the processes that associate with a single cone, and as many as five have been identified. The cone illustrated in Fig. 2a has two phagosomes within the processes (arrows). They can be distinguished readily from melanin granules, which are usually oval and do not have a disk-like substructure. Each phagosome consists of a group of cone disks still enclosed within the cell membrane of the outer segment (Fig. 2b). The portion of cone outer segment is itself surrounded by the membrane of the epithelial processes (Fig. 2b, arrows). The disks of this phagosome (Fig. 2b) have maintained their original orientation while the disks of the other phagosome have rotated 90°. The phagocytosed disks stain more deeply than the disks of the intact outer segment.

The processes containing the phagosomes are continuous with the processes that surround the outer segment along its outer half (Fig. 2a and c). Here, the processes are directly involved in phagocytosing the tip of the outer segment. A single process may bifurcate so that it surrounds the tip (Fig. 2c, arrow). Figure 2d shows the tip of another outer segment where a pigment epithelial process has extruded a pseudopod (arrow) which traverses the outer segment and isolates a group of 40 disks from the remainder of the outer segment. Other stages in the phagocytic process also have been seen.

Phagocytosis of portions of human cone outer segments is similar to that found in human and Rhesus monkey rod outer segments. For both classes of photoreceptor the pigment epithelial processes intermittently surround and then engulf a group of disks from the tip of the photoreceptor<sup>12,13</sup>. This suggests that human cone disks also must be continuously renewed, although the mechanism of renewal may differ from that of the rod. This conclusion is supported by the observation



Fig. 1 Light micrograph of two cones. Fixation took place in 3% glutaraldehyde-paraformaldehyde, 0.2 M Na cacodylate buffer with the addition of 1% sucrose, CaCl<sub>2</sub> and KCl, pH 7.4 for 2–3 h at room temperature. This was followed by post fixation in 2% osmium tetroxide in Na veronal acetate buffer with the addition of sucrose for 2 h and then by block staining in 2% uranyl acetate with Na H maleate buffer for 2 h (dark). The tissue was dehydrated in graded acetone and embedded in Araldite. The arrows point to inclusions within the pigment epithelial processes, which are presumed to be phagosomes. Bar equals 5.0µm.



Fig. 2 a, Electron micrograph montage of a cone. Slender pigment epithelial processes, containing two phagosomes (arrows), extend to the outer segment tip. This section is somewhat atypical in that the processes are fewer in number and thinner than in the average section. b, Enlargement of phagosome closest to outer segment tip. Pigment epithelial cell membrane (arrows) surrounds a group of 62 disks. c, Enlargement of outer segment tip showing birfurcation of a pigment epithelial process (arrow). d, Outer segment tip of a different cone. A pseudopod from a pigment epithelial process extends across the outer segment isolating the 40 terminal disks. Bars equal 0.5  $\mu$ m (a, b, c); 0.25  $\mu$ m (d).

that Rhesus monkey cone outer segments regenerate after having been damaged by separation from the pigment epithelium<sup>14</sup>.

Finally, that continuous renewal of cone disks may be general in mammals is suggested by a recent description of disk shedding by the cone-like photoreceptors of diurnal tree squirrels<sup>15</sup>.

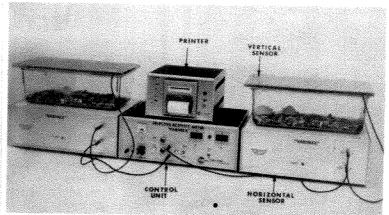
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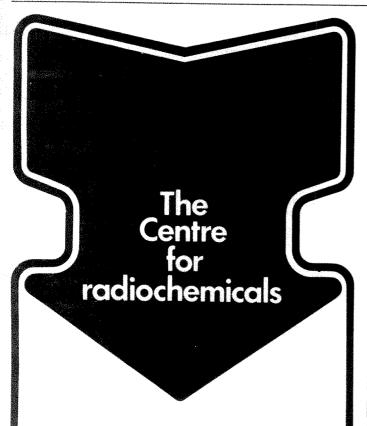
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### Antigenic specificity of retinal pigment epithelium and non-immunological involvement in retinal dystrophy

Organ specific antigens have been demonstrated in the retina proper and particular attention has been paid to their relation to eye disease1-7. The antigenicity of the photoreceptors has

also been demonstrated8 and its implication in the pathological process of retinal dystrophy in experimental animals and in man has been lately under evaluation 1-10. In the case of the retinal pigment epithelium (RPE), however, no conclusive evidence of its antigenicity or immunological involvement in ocular disease has so far been produced. Nevertheless this layer fulfils an important physiological role11-13 and its primary involvement in retinal dystrophy has been emphasised14-17

This study was carried out to determine the antigenicity of the RPE and to determine whether any specific autoallergic reaction to RPE may be found in retinal dystrophy in rats. RPE obtained from normal albino rabbits was grown as cellular monolayers in tissue culture; the tissue was freezedried and stored. A Dunkin Hartley adult guinea pig was immunised by six injections of 4 mg of the freeze-dried material given subcutaneously at weekly intervals together with Freund's complete adjuvant. The animal was killed at the end of the experiment and the serum was stored at -20° C until used. Several Cryostat sections of four normal eyes from albino rabbits and several primary cultures of RPE from 12 normal eyes of albino rabbits and the same number of normal eyes from Wistar albino rats, were studied by indirect immunofluorescence technique, using fluorescein-labelled anti-guinea pig serum. Cryostat sections and primary cultures treated first with normal guinea pig serum and then with fluoresceinlabelled anti-guinea pig serum were used as controls. Antibodies to RPE were present in the sensitised guinea pig serum (Fig. 1), which reacted intensely with cultured rabbit RPE and somewhat weakly with cultured rat RPE; both cytoplasmic and membranous fluorescence were observed suggesting that the antigens are located on the surface membrane as well as intracellularly. Whether or not these represent two distinct antigen systems requires further study. In whole cryostat

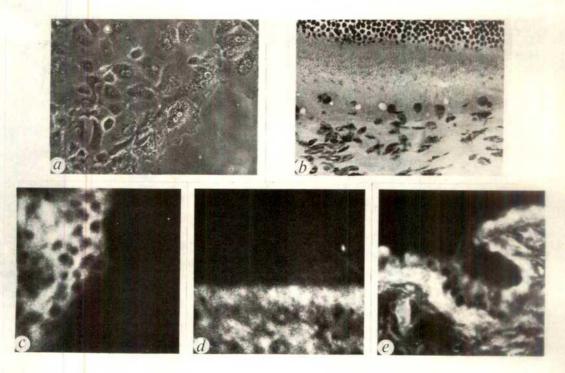


Fig. 1 Indirect immunofluorescence study of Cryostat ocular sections and RPE cultures from albino rabbits. The sections of sensory retina, RPE and choroid were cut in a tangential oblique plane; ciliary processes were sectioned anteroposteriorly. Primary explants of rabbit RPE, grown on glass, were cultured in medium 199 (Wellcome) and heat-inactivated rabbit serum (30%) to which fresh glutamine (Bio Cult) was added (300 mg per 1000 ml). Cultures on their glass coverslips were thoroughly rinsed in warm (37° C) balanced salt solution. Both Cryostat sections and primary cultures were fixed in ether/acetone before sequential incubation with sensitised or with normal guinea pig serum and fluorescein labelled anti-guinea pig serum (Wellcome); they were incubated at room temperature for 30 min and washed in a bath of phosphate buffer saline (pH 7.6) for 30 and 45 min. respectively. All sera were diluted 1:10, a, 3 day-old primary culture of rabbit RPE (phase contrast, × 82.5); b, cryostat section of retina, RPE and choroid (haematoxyin and eosin × 72.5); c, indirect fluorescence localisation in 3-day old primary culture of rabbit RPE (× 99); d, cryostat section of retina, RPE and choroid. Note the intensity of RPE fluorescence outlined as an approximately horizontal band between the non-fluorescent retina and the underlying fluorescent choroid 198); e, cryostat section of ciliary processes. Note the convoluted fluorescent layer of the pigmented epithelium upon the surface of which the non-pigmented ciliary epithelium can be observed with a background negative fluorescence. The stroma presents a weak positivity  $(\times 198).$ 

sections of rabbit eyes no fluorescence was seen in the sensory retina including photoreceptors, thus further suggesting that the RPE which develops from the outer layer of the secondary optic vesicle is antigenically distinct from the sensory retina, especially the photoreceptors, which develop from the inner layer of the secondary optic vesicle.

A positive fluorescence was, however, observed in the RPE and pigmented layer of the ciliary epithelium and also in the extravascular choroidal tissue. As the pigmented epithelium of the ciliary body is the forward continuation of the RPE it is understandable why this tissue cross-reacted with the anti-RPE serum; the fluorescence of the choroid remains unexplained but it suggests that extravascular choroidal tissue has some antigenic material in common with the RPE. As growth patterns of cultured RPE are quite different from those of cultured choroidal mesenchyme and as extreme care was taken to obtain pure RPE for tissue culture it seems highly unlikely that the materials we used for sensitising guinea pigs were contaminated with choroidal tissue, but it is a possibility which is difficult to rule out.

To investigate whether autoantibodies to RPE could be demonstrated in cases of rat retinal dystrophy, flat preparations of RPE were obtained from Wistar albino rats and inbred PETH dystrophic rats of comparable age groups. Serum was obtained from two groups of inbred PETH dystrophic rats: first, animals of approximately 3 weeks of age showing early retinal degeneration and, second, animals of 1.5–2 months of age showing marked retinal degeneration. Indirect immuno-fluorescence study using fluorescein-labelled anti-rat immuno-globulin serum failed to demonstrate any antibodies against RPE in dystrophic animals.

For the first time it has been possible to demonstrate the antigenicity of the RPE and it seems that the antigen present within it is quite distinct from those present in the visual cells of the retina, but no evidence was obtained that this plays a role in retinal dystrophy of the rat.

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### X-ray dose response for mutation to fructose utilisation in cultured diploid human fibroblasts

THE majority of studies relating to somatic cell mutation in cultured mammalian cells have been performed with established (heteronuclear) mammalian cells2. There are important karyotypic and metabolic differences, however, between cultured heteronuclear cells and mammalian cells in vivo and mutation in cultured diploid (homonuclear) cells may be more relevant to the in vivo situation. Mutation, in the broadest sense of the term1, has been studied in cultured diploid cells2-9 and there is evidence, from the work of Albertini and DeMars2 with 8-azaguanine resistant (Agr) variants of diploid human fibroblasts, that it may be possible to quantify radiation-induced mutation in diploid mammalian cells. Here we report that the utilisation of fructose, in place of glucose, as the major carbon and energy source, was used as a phenotypic character for mutation studies with a culture of diploid human fibroblasts. Although not yet fully understood, the fructose-utilisation mutation system does circumvent some of the problems of mutation expression time associated with the selection of •Agr variants of cultured mammalian cells.

Early passage HF10 diploid human fibroblasts (6-25 generations in vitro) grew to confluency when the hexoses D(+) galactose or D(+) mannose replaced D(+) glucose as the major carbon and energy source in Eagles minimal essential medium (MEM) containing 10% dialysed foetal calf serum (MEMFCS). The hexose,  $\beta D(-)$  fructose, did not replace

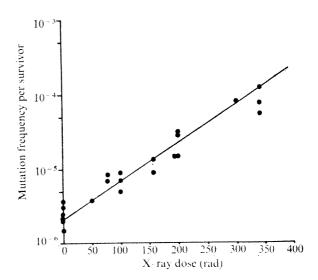


Fig. 1 X-ray dose-response for induction of fructose-utilising variants of 6-25 generation HF10 diploid human fibroblasts. Each point represents between ten and twenty variant clones scored. HF10 diploid human fibroblast cultures were initiated from 3 embryonic limb tissue and maintained in monolayer culture at 37° C using methods previously described12. for mutation induction experiments were trypsinised from culture vessels during the exponential phase of growth and resuspended in hexose-free growth medium (Eagles minimal essential medium (MEM) plus 10% foetal calf serum, Flow Laboratories, Irvine, Scotland). Cells were irradiated in suspension with varying doses of 250 kV X rays, dose rates varying between 50 and 250 rad min<sup>-1</sup>. Immediately after irradiation  $2 \times 10^5$  cells were added to each 9 cm tissue culture grade Petri dish (Sterilin, Richmond, Surrey, England) which contained 10 ml glucose-free MEM containing 1 mg ml $^{-1}$   $\beta D(-)$  fructose (Sigma, Kingston, Surrey, England) plus 10% dialysed foetal calf serum. The serum batch used in these experiments was dialysed for 4 h at 4° C against 100 volumes of 0.85° NaCl. Petri dishes were incubated at 37° C in CO2 enriched air for 14 d and variant clones arising in the fructose medium were stained for 1 h with 0.25% Azur A stain (Searle Diagnostic, High Wycombe, England). Mutation frequencies after X-irradiation were calculated with respect to the fraction of cells surviving each dose and the dose-response curve drawn by eye through the data points.

Table 1 Distribution of clones utilising fructose amongst Petri dishes containing irradiated and non-irradiated HF10 cells.

$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	futation frequency per survivors 3.1 × 10-6 7.1 × 10-6 2.8 × 10-6 7.5 × 10-8
---	--

\*2×105 cells per 9 cm plastic Petri dish.

†Figures in parentheses are expected Poisson distributions of total Frue+ clones.

Minimum estimate not corrected for efficiency of recovery of clones<sup>2</sup>.

glucose as a carbon and energy source and HF10 cells degenerated in fructose MEMFCS at the same rate as they did in hexose-free MEMFCS. Limited growth of HF10 cells occurred in hexose-free MEMFCS unless the foetal calf serum (FCS) added to the growth medium was dialysed to reduce the concentration of glucose in the serum.

With the optimum number of HF10 cells  $(1 \times 10^5-2 \times 10^5)$  in 9 cm tissue culture grade plastic Petri dishes containing 10 ml fructose MEMFCS, fructose-utilising (Fruc<sup>+</sup>) clones arose at a spontaneous frequency of approximately  $10^{-6}$ . As no clones were seen when fructose was omitted from the medium, other medium or serum components are of themselves insufficient. This observation contrasts with the work of Varshaver et al.<sup>9</sup> who found that human diploid fibroblasts selected by their ability to grow in a fructose medium were probably dependent for growth on other medium or serum components.

Three Fruc<sup>+</sup> clones isolated from HF10 cultures were karyotypically normal, had stable phenotypes during ten generations *in vitro* and were able to grow to confluency with glucose or fructose as a major carbon and energy source. Two Fruc<sup>+</sup> variants that were tested were also biochemically distinguishable from the parental strain HF10 cells; both Fruc<sup>+</sup> clones exhibited a marked increase in the rate of uptake of <sup>14</sup>C-fructose and <sup>14</sup>C-glucose (R.C., and W.K.M., unpublished) Preliminary mutation fluctuation tests<sup>4,7,9</sup> showed that Fruc<sup>+</sup> phenotypes arose in a random fashion in parallel unselected HF10 cultures, independent of the selective growth conditions. These observations alone, however, are insufficient to enable us to comment upon the genetic basis of Fruc<sup>+</sup> variants of diploid human fibroblasts.

Preliminary experiments with X-irradiated cultures of HF10 showed that the frequency of the Fruc+ phenotype amongst survivors was greater than that observed in unirradiated controls. Mutation expression time experiments were performed, with varying periods of post-irradiation growth in non-selective medium (glucose MEMFCS) before transfer of the cells to fructose MEMFCS. The maximum yield of radiation-induced Fruc+ clones was found when the cells were transferred to fructose medium immediately after irradiation, showing that the Fruc+ phenotype was fully expressed at zero expression time<sup>10</sup>.

The X-ray dose-response curve for the induction of the Fruc+ phenotype, when irradiated cells were plated in fructose medium immediately after irradiation, is shown in Fig. 1. The mutant frequency per surviving cell was cumulative and increased approximately exponentially with dose from  $2.0 \times 10^{-6}$  at zero dose to  $1.0 \times 10^{-4}$  at 350 rad. There was no absolute increase, however, in the number of mutant clones per Petri dish (Table 1), and this ratio showed no significant change with dose. This result could indicate a chance coincidence, that is, that the rates of cellular mutation and inactivation were approximately the same over this dose range. Another possible explanation of the findings is that the Fruc+ variants were more radioresistant than the parental strain, and that the radiation. rather than the growth medium, was selecting the variant clones. This seems unlikely because cell cultures grown from two of the Fruc+ variants had X-ray survival curves similar

to that of an HF10 parental culture of comparable generation number (Fig. 2). A further possibility that the Fruct variant detected in irradiated cultures were not induced by the radiation but arose spontaneously during the growth and division cells surviving the radiation is also unlikely as the cell were plated into selective medium immediately after irradiation thus allowing no time for spontaneous Fruct variants to arise from the growth and division of non-variant surviving cells. The preliminary conclusion is therefore that Fruct variant were induced by ionising radiation and that the dose-responsibility was approximately exponential.

Albertini and DeMars<sup>2</sup> found that, like the Fruc<sup>+</sup> variant reported here, Ag<sup>r</sup> variants of diploid human fibroblasts als did not show an absolute increase in number after X-irradiatio but did show a dose-dependent increase in mutant frequenc per surviving cell. An essentially similar result has been of tained independently in this laboratory with Ag<sup>r</sup> variants c HF10 cultures but because a post-irradiation growth period c ~3 d in non-selective medium was necessary for optimur recovery of Ag<sup>r</sup> clones, the interpretation of any dose-respons curve must be complicated by the problem of spontaneou mutation of survivors. The Fruc<sup>+</sup> variants, with an apparen zero mutation expression time, may therefore provide a technically more straightforward mutation system.

If the failure to demonstrate an absolute increase in mutan number with dose reflects the relatively poor mutagenicity of X rays in diploid human fibroblasts, N-methyl-N-nitro-N nitrosoguanidine (MNNG), which has been shown to be a more potent mutagen that X rays in cultured heteronuclea mammalian cells<sup>13</sup>, should give an absolute increase in mutan number with dose. Preliminary experiments with MNNC suggest that this hypothesis is correct and an absolute increase in mutant number with dose has been observed for both Agr variants<sup>2</sup> and Fruc+ variants (R.C., and W.K.M., unpublished) of diploid human fibroblasts.

Although some doubt must remain over mutation experiments that do not demonstrate an absolute increase in mutan

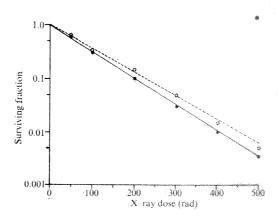


Fig. 2 X-ray survival curves of 25 generation cultures of. . HF10 parental strain and, O, a derivative fructose-utilising variant, Fruc-2. X-ray survival data were obtained using the 'feeder cell' technique previously described's.

Table 2 Forward mutation rates in mouse germ cells in vivo and diploid human fibroblasts in vitro after single exposures to X rays at dose rates 50-250 rad min-1

4 William Control of the Control of	Mean induced	**************************************	
Mutation system	mutation rate	Dose	Reference
	per locus	range	
	per rad		
Mouse spermatogonia	$2.64 \times 10^{-7}$	0-300 rad	14*
(mean of seven loci)	•		v
Mouse oocytes	$1.08 \times 10^{-7}$	0-50 rad	14*
(mean of seven loci)	$3.77 \times 10^{-7}$	0-200 rad	
,	$5.41 \times 10^{-7}$	0-400 rad	
Diploid human fibroblasts	$1.13 \times 10^{-9}$ †	0-75 rad	2
$(Ag^r)$	$7.49 \times 10^{-8}$ †	0-125 rad	
	$6.87 \times 10^{-8}$ †	0-150 rad	
	$2.16 \times 10^{-7}$ †	0-250 rad	
Diploid human fibroblasts	$4.00 \times 10^{-8}$	0-100 rad	Present work
(Fruc <sup>+</sup> )	$1.24 \times 10^{-7}$	0-200 rad	
- *	$2.10 \times 10^{-7}$	0-343 rad	

<sup>\*</sup>Data also available on mutation induction in mouse germ cells at lower dose rates.

†Calculated as mutation rate per survivor after correction for efficiency of recovery of clones.

‡Calculated as mutation rate per survivor with no correction for efficiency of recovery of clones.

number with dose, the results of Albertini and DeMars<sup>2</sup> and those reported here suggest that the Agr and Fruc+ mutation systems may be useful for quantitative studies of mutation in cultured diploid human fibroblasts. It is also possible that the cumulative X-ray dose response observed with both systems is a true reflection of radiation induced mutation in cultured diploid human fibroblasts. A more complete study of the genetic basis of somatic cell variants and the phenotypic expression of induced mutation with these and other mutation systems, however, is required before dose-response curves for mutation can be interpreted with confidence.

With these experimental limitations in mind, it may be useful to compare X-ray mutation rates obtained in vitro in cultured diploid human fibroblasts and in vivo in mouse germ cells (Table 2). For dose rates between 50 and 250 rad min<sup>-1</sup> there are insufficient data to determine the shape of the doseresponse curve for mutation in mouse spermatogonia, although there is evidence that mutation induction per rad is nonlinear<sup>14</sup>. In mouse oocytes<sup>14</sup> and with both mutation systems in diploid human fibroblasts there is fairly clear evidence of a nonlinear cumulative dose-response. Mutation rates for Agr and Fruc+ variants of diploid human fibroblasts seem to be closely similar. For experiments at comparable dose rates and doses of 200-300 rad, the in vitro mutation rates in diploid human fibroblasts are also similar to in vivo mutation rates in both mouse spermatogonia and mouse oocytes. Although such numerical similarities could be misleading, it is hoped that further studies on radiation-induced mutation in cultured diploid somatic cells may help to elucidate the underlying cellular mechanisms involved in mutagenesis of normal mammalian cells in vivo.

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### TRH: a possible mediator of thermoregulation

FELDBERG and Myers1 have reported that intraventricular injections of amines produce changes in the rectal temperature of unanaesthetised cats. They noted that more than 50  $\mu g$  noradrenaline produced temporary hypothermia • whilst 200 μg 5-hydroxytryptamine (5HT) induced a sustained hyperthermia. These results, coupled with earlier findings that these amines were concentrated in the hypothalamus2.3, led them to speculate that the regulation of body temperature in the cat may be mediated by the release of noradrenaline and 5HT within the hypothalamus.

Observations by other workers have raised the possibility that the ratio of cations in the hypothalamus or the release of prostaglandin E15 from the same structure may also be important for the chemical mediation of body temperature. This report concerns the effects produced when small quantities of releasing hormones, peptides also derived from hypothalamic tissue, were injected directly into the cerebral ventricles of conscious cats.

Sterile solutions of thyrotropin releasing hormone (TRH), luteinising hormone releasing hormone (LRH) and melanocyte stimulating hormone release-inhibiting factor (MIF) were injected into conscious cats in 0.2 ml artificial cerebrospinal fluid by a cannula previously implanted in the lateral ventricle. LRH and MIF were without effect on body temperature at doses up to 5  $\mu$ g. In contrast, similar doses of TRH produced a dose-related hypothermia (Fig. 1). Although the minimal effective hypothermic dose varied between individual animals, in some cats as little as 3 ng TRH produced 0.5° C fall in body temperature. At doses greater than  $0.5~\mu g$ , TRH (in common with hypothermic doses of noradrenaline and calcium) produced a sedative or quieting effect. In addition, all three peptides frequently produced defaecation and urination whilst TRH alone induced profuse salivation in some animals.

The doses of TRH needed to induce hypothermia compare favourably with the calculated TRH content of the hypothalamus<sup>6,7</sup> and stand in contrast to the fact that the quantity of noradrenaline needed to provoke hypothermia (50 µg) greatly exceeds the endogenous hypothalamic concentration of that amine2.8. Indeed, TRH seems to be the most potent hypothermic agent yet reported (Fig. 2) comparable in its potency to the hyperthermic activity reported by Milton and Wendlant<sup>5</sup> for prostaglandin E<sub>1</sub>.

The comparison between noradrenaline and TRH is also interesting from other points of view. Noradrenaline has been reported to stimulate the synthetase enzyme responsible for TRH production whilst 5HT seems to inhibit this same enzyme9. Calcium ions, known to be critically involved in the release of noradrenaline from nerve endings10,11, themselves produced hypothermia4 whilst imipramine, an antidepressant drug which inhibits the reuptake of noradrenaline into nerve endings, also produces

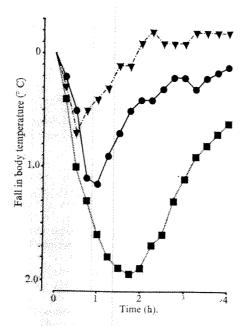


Fig. 1 Hypothermia produced in individual cats by the intraventricular injection of.  $\nabla$ , 0.05  $\mu$ g;  $\bigcirc$ , 0.5  $\mu$ g;  $\square$ , 5  $\mu$ g TRH. Drug injected at time zero.

hypothermia in cats12. Thus, it is possible to explain the hypothermia produced by all of these agents in terms of the TRH-induced hypothermia reported here.

Cooper et al.13 noticed a species difference to the effects of amines on body temperature when they found that intraventricular noradrenaline produced hyperthermia in rabbits. This observation has been confirmed by Jacob14 and is in keeping with the observation that intraventricular desipramine also produces hyperthermia in this species12 Preliminary observations in rabbits have shown that doses of 0.5 or 1.0 µg TRH injected intraventricularly produce a hyperthermia of 0.6-1.0° C.

There is speculation amongst psychiatrists as to whether TRH is an effective antidepressant agent 15,16,17. My results are consistent with the suggestion that TRH may possess antidepressant activity and may add a new dimension to

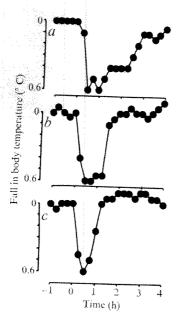


Fig. 2 Hypothermia produced in individual cats by a artificial CSF containing 20 mM excess  $Ca^{2+}$ ; b, 50  $\mu g$  noradrenaline; c, 10 ng TRH. All drugs were injected at time zero.

the amine theory of affective disorders18 in that the amines may act indirectly by their effects on TRH production. Whether this is proven or not, the present results certainly indicate that TRH needs careful evaluation as a possible modulator of mammalian thermoregulation.

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### A merozoite vaccine effective against Plasmodium knowlesi malaria

Plasmodium knowlesi malaria is uniformly fatal in rhesus monkeys1. If infections are initially controlled with drug therapy, however, a degree of immunity can be induced associated with periodic relapse and low grade infection after repeated challenge. In such resistant monkeys each recurring infection is attributable to a distinct parasite variant recognisable by the schizont-infected cell agglutination (SICA) test2. Antigenic variation therefore seems to be an important mechanism determining chronicity of infection3 and complicates any attempt to achieve effective immunoprophylaxis'. Rhesus monkeys have been vaccinated with formol-treated\*, or freezethawed' erythrocytic schizonts of P. knowlesi or fractions of these parasites<sup>8,9</sup> in complete (FCA) or incomplete (FIA) Freund's adjuvant. Such vaccination reduced mortality to about 50% after challenge with the homologous variant; in surviving monkeys parasitaemia reached 3-10%, but parasites were completely eliminated after about 2 weeks and some animals were then resistant to heterologous challenge7. When the first challenge variant was known to differ from that used for immunisation, all monkeys suffered fatal infections'. The protection achieved always required the use of complete adjuvant, but this alone was inactive.

Table 1 Infection of normal rhesus monkeys with 10<sup>4</sup> P. knowlesi parasites (control infections for challenge of immunised animals)

***	D.44	Mariana manaitanniat	Death	Drug cure
Parasite*	Patent	Maximum parasitaemia†		
(variant)	(day)	(per 10 <sup>4</sup> erythrocytes)	(day)	(day)
W(1)	4	1800	8	
W(1)	3	670	10	
$\mathbf{W}(1)$	3	320		5
W(3)	5	3000	9	
W(3)	3	2000	7	
W(42)	3	130		6
W(12)	4	1100	8	
N(1)	4	500		6

\*W, Walter Reed strain; N, Mill-Hill ('Nuri') strain of *P. knowlesi*. Numbers denote distinct variants differentiated by the SICA test<sup>2,17</sup>. †Parasitaemia on the day before death or drug cure.

Studies on the mechanism of acquired malarial immunity in vivo<sup>10</sup> and in vitro<sup>11</sup> indicated that protective antibody does not affect intracellular parasites, but interrupts the cycle of development at the stage when merozoites are released into the plasma. In addition, immune serum agglutinated free merozoites and prevented their attachment to receptors present on the red cells of susceptible species These findings suggested an important role for merozoite antigens in specific malarial immunity and prompted development of an in vitro method for isolating extracellular blood stage merozoites. Here we describe the use of these preparations for vaccination of rhesus monkeys against P. knowlesi malaria.

Monkeys (Macaca mulatta) were of either sex and weighed about 3 kg. Parasites were originally obtained from sources indicated in Table 1. Variants of P. knowlesi were differentiated by the SICA test<sup>2</sup>. Merozoite preparations (W1 variant) were isolated as described<sup>14</sup> except that phytohaemagglutinin was used in place of antiserum to agglutinate parasitised and normal red cells. The yield of merozoites was approximately 2×10<sup>10</sup> per ml of cultured erythrocytic schizonts and contamination with parasitised red cells was less than 0.1%. Two monkeys were immunised three times during 7 weeks with 1.3×10<sup>8</sup> mature schizonts (W1 variant) in FCA as controls for the schizont contamination of the merozoite vaccine. Freshly isolated merozoites suspended in 1 ml medium 199 (Burroughs-Wellcome) were emulsified with equal volumes of FCA or FIA and administered by intramuscular injection to six monkeys as shown in Table 2. Immunisation never produced a detectable infection. Immunised animals were challenged by intravenous injection of 10<sup>4</sup> or 10<sup>5</sup> immature schizonts of known strain and variant specificity on days shown in Table 2. Thick and thin blood films were examined five or more times weekly from the time of challenge. Equivalent challenge infections were administered simultaneously to normal rhesus and all suffered progressive infections. Five out of eight of the control animals died within 7–10 d and three others were cured on day 5 or 6 when the level of parasitaemia indicated a fatal outcome.

Three merozoite immunised animals were challenged first with the same variant (W1) as used for immunisation. No infection was detectable in two of these monkeys (Mz 12, 10, Table 2) and subinoculation of 5 ml of blood from one into a normal rhesus on the 11th d after challenge revealed no viable parasites (Mz 10, Table 2). One monkey (Mz 16, Table 2) challenged with W1 variant developed parasitaemia for 6 d with a maximum of 0.04%. A schizont-immunised rhesus challenged with the homologous variant showed parasitaemia for 11 d with a peak of 1.3% (cf. ref. 7). Three other merozoite immunised animals were challenged first with a heterologous variant (W3) of P. knowlesi. All showed parasitaemia which lasted for 8-12 d and did not exceed 0.07-1.5% in the individual monkeys (Mz 14, 15 and 17, Table 2). A schizont-immunised animal challenged first with the W3 variant developed a fatal infection indistinguishable from that in unimmunised controls (cf. ref. 7) (Fig. 1). Five of the six merozoite immunised animals were subsequently challenged on eight occasions during a period of up to 10 months with four distinct variants and a different laboratory strain of P. knowlesi (Table 2). Seven of these challenges did not produce a detectable infection and viable parasites could not be demonstrated by subinoculation from Mz 10 (Table 2) after challenge with three different variants during a period of about 2 months. This animal finally developed a patent infection lasting 9 d with maximum parasitaemia of 0.02% when challenged with the W1 variant 33 weeks after initial vaccination (Table 2).

In contrast to what is observed after immunisation with parasitised cells<sup>5-9</sup> or repeated infection initially controlled with drug therapy<sup>15,16</sup> these results show that challenge after vaccination with merozoites derived from a single serological variant of *P. knowlesi* leads to frequent absence of detectable parasitaemia (nine out of fourteen instances); occasional sterile immunity (shown by subinoculation in two

Neg

Table 2 Merozoite (W1 variant) vaccination of rhesus monkeys against P. knowlesi malaria Monkey Vaccination (W1) Challenge (104 parasites)\* no. Parasitaemia Subinoculation **FCA**† FIA† Day‡ Parasite Max per (day)§ Days patent (day) (day) 104 RBC Neg Mz 12  $5 \times 10^8$  $2 \times 10^9$  $1 \times 10^9$ 98 W<sub>1</sub> (1) (75)S (85) Mz 10  $5 \times 10^9$  $6 \times 10^9$  $5 \times 10^8$ Neg 93 Neg (14)(37)127 W12 Neg 232 1 (259) 242-251 280 Neg 1 (124)¶ 131-137 Mz 16  $6 \times 10^8$  $9 \times 10^8$  $2 \times 10^9$  $5 \times 10^9$ Neg 7 (98)148 Mz 14  $6 \times 10^8$  $9 \times 10^8$  $2\times10^9$ 76 81--- 89 Neg (14)(62)118 83-95  $6 \times 10^8$  $9 \times 10^8$ Mz 15  $2 \times 10^{9}$ 76 Neg 28 118 (62)(14)118;131-139 Mz 17  $6 \times 10^8$  $2 \times 10^{9}$  $5 \times 10^9$ 

148

(1)

(14)

<sup>\*</sup>For details of parasites see footnote Table 1.

<sup>†</sup>No. merozoites in Freund's complete (FCA) or incomplete (FIA) adjuvant.

Days refer to time after first vaccination. \$Subinoculation of 5 ml blood from vaccinated into normal rhesus on day shown. S, No infection; I, infection in recipient.

Challenge with 10° parasites.

Infection in recipient of blood was due to a P. knowlesi variant other than W1.

instances); parasitaemia of short duration (6-12 d) and relatively low intensity (maximum 0.02-1.5%) when patent infection did occur (five out of fourteen instances); resistance to several serological variants different from that used for immunisation. The available data suggest that the various schedules of vaccination employed (Table 2) provided comparable protection; we are now attempting to establish whether adjuvant is essential for merozoite vaccination. The number of merozoites was chosen arbitrarily; a smaller number might be effective, but at the level used, the in vitro cultures provide about 20 immunising doses per ml parasitised red blood cells. The induced immunity seems to be predominantly species specific since 3 merozoite immunised animals challenged with 10° P. cynomolgi bastianellii parasites developed chronic patent infections, although maximum parasitaemia was lower than in normal monkeys (Table 3). P. knowlesi infections controlled by drug therapy do not cross immunise against P. cynomolgi bastianellii<sup>17</sup> which produces chronic, non-fatal malaria in rhesus monkevs.

We conclude that merozoite vaccination protects rhesus monkeys against initial homologous or heterologous variant infection with erythrocytic stages of the normally lethal . P. knowlesi parasite. Vaccinated animals once challenged are also resistant to subsequent infection with other variants or a different laboratory strain of P. knowlesi. Merozoite vaccination induces immunity far greater in degree and significantly broader in specificity than previously achieved by immunisation or repeated infection. This form of vac-

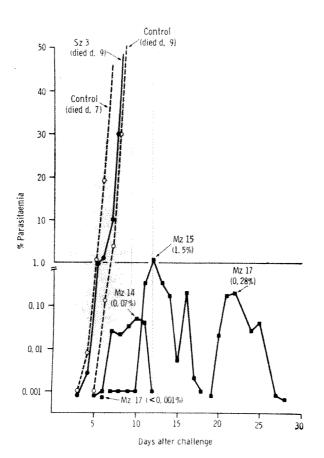


Fig. 1 Course of parasitaemia in two normal rhesus monkeys, one immunised with schizonts (Sz 3), and three immunised with merozoites (Mz 14, 15 and 17, Table 2). All animals were challenged with 10<sup>4</sup> P. knowlesi parasites (Mz 14, 15 and 17, Table 2). (W3 variant). Sz 3 had been vaccinated with a total of  $1.3 \times 10^8$  erythrocytic schizonts (W1 variant) on days 1, 41 and 77 and was challenged on day 91. To illustrate the course of low-grade infections in the merozoite immunised animals, parasitaemia (vertical axis) is shown on a logarithmic scale between 0.001 and 1.0%.

Table 3 P. cynomolgi bastianellii challenge (105 parasites) of normal controls and monkeys vaccinated with P. knowlesi merozoites

Monkey 389-normal 391-normal	Day of challenge*	Pre-patent period (d) 5 4	Maximum parasitaemia per 104 RBC 1200 1250	Duration of patent infection (d) > 80 > 80
Mz10(†)	323	12	68	> 80
Mz14(†)	142	4	400	> 80
Mz15(†)	142	5	550	> 80

\*Day after initial vaccination on which P. cynomolgi bastianellli challenge was administered.

†See Table 2 for previous immunisation and challenge of merozoite vaccinated animals.

cination apparently stimulates a specific response to antigens present on a wide spectrum of P. knowlesi parasites, but poorly inductive, during natural malaria infection. Our observations are significant with regard to the development of an effective vaccine against human malaria in view of the probable existence of immunologically distinct strains of P. falciparum18,19 and the established genetic diversity of this parasite in West Africa20.

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### Excystment by sporozoites of malaria parasites

IT is widely assumed that the mature oocyst of the malaria parasite bursts and liberates the sporozoites en masse1 although studies on the intestinal coccidians have clearly shown that the sporozoites escape through the wall of the sporocyst, a structure analagous to the oocyst of malaria parasites, by way of specialised pores2-4. During a scanning electron microscope study of the sporogonic cycle of the rodent malaria parasite Plasmodium yoelii nigeriensis in the vector Anopheles stephensi, evidence has been obtained which suggests that excystment of the sporozoite may occur by a method differing from both those described above.

Examination of oocysts on a very heavily infected gut prepared 11 d after infection revealed the presence of small holes, 0.25-0.65 µm in diameter, in the wall of some cysts (Fig. 1a). Furthermore these perforations were limited to areas some 15 µm across on oocysts 40 µm in diameter. The holes were found with increasing frequency towards the centre of this region, where larger perforations appeared. Sporozoites could be seen emerging individually through these holes. While this might have represented the early stages of the total destruction of the oocyst, evidence to the contrary was provided by the occurrence of empty oocysts in which only small regions of the cyst wall were damaged (Fig. 1b). The basal lamina of the mosquito gut epithelium lay as a diaphanous netting over the surface of most oocysts, but frequently could not be detected at all. Should this reflect the in vivo condition the basal lamina can be considered little obstacle to the escaping sporozoites.

These observations suggest that sporozoites escape through the oocyst wall by small perforations which become enlarged as emergence progresses (Fig. 1a and b). In some instances this process may be traumatic enough to destroy the oocyst. Further, it is clear there is no organelle in the wall of malaria parasite oocysts through which the sporozoites emerge, comparable to the pore occupied by the Stieda body in the sporocyst wall of intestinal coccidians.





Fig. 1 a, Scanning micrograph showing sporozoites escaping from perforated oocyst of Plasmodium yoelii nigeriensis on the midgut of Anopheles stephensi. The infection was maintained for 11 d at 25° C and 80% relative humidity. The specimen was dissected into Schneider's Drosophila medium and rinsed briefly. Following fixation in 3% glutaraldehyde in 0.1 M phosphate buffer pH 7.4 the specimen was washed in 0.15 M phosphate buffer and postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer. Dehydration was in acetone. Samples were dried in carbon dioxide (using the critical point method) and gold coated in a 'sputter coater' (×°60). b, Micrograph of oocyst following the escape of the sporozoites. (×°12).

While it is recognised that the preparations examined bore unusually high densities of oocysts, and the consequent pattern of sporozoite release need not be identical to that normally seen, it is still necessary to explain both the direction and the restricted path of migration of the sporozoites through the oocyst wall. The breakdown of the oocyst could be a passive process determined by areas of weakness in the cyst wall. Alternatively the sporozoites may penetrate the oocyst actively, their orientation to areas of cyst wall that lie adjacent to the haemocoele being determined by some biochemical gradient between the surrounding haemocoelomic fluid and midgut epithelium of the

mosquito. In the present study the intimate contact of the adjacent oocysts could severely limit these areas of the cyst wall in contact with the haemocoelomic fluid.

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### Evidence for energy-dependent accumulation of paraquat into rat lung

THE herbicide paraquat (N,N'-dimethyl 4,4'-bipyridilium) can produce widespread oedema and fibrosis in the human lung after accidental ingestion1-3. In those cases where death occurs after several weeks, there are no apparent pulmonary changes during the first few days following ingestion. Animal experiments in a variety of species have shown the lung to be the major target organ4-6. After administration of paraquat to animals, the lung has a high initial concentration and retains paraquat7-This retention appears to be related to the development of lung damage<sup>7</sup> (L. L. Smith and M. S. Rose, unpublished work). The mechanism of retention of paraquat by the lung is at present not understood.

We have demonstrated an energy-dependent accumulation of paraquat in slices of rat lung. This process may account for the retention of paraquat in the lungs of many species. Diquat (N,N'-ethylene 2,2'-bipyridilium), a herbicide closely related in structure and properties to paraquat, is not actively accumulated by lung slices, is not retained by the lung in vivo7.8 and does not damage the lung10 (L. L. Smith and M. S. Rose, unpublished work).

Male Alderley Park (Wistar derived) specific pathogen free rats (body weights 170-200 g) were killed with halothane. The lungs were removed and slices prepared (20-70 mg wet weight) at room temperature. Only slices with two cut surfaces were used. Incubation was carried out at 37° C in 3.0 ml Krebs Ringer phosphate<sup>11</sup> containing 200 mg% (w/v) glucose, 0.1 μCi of methyl-14C-paraquat or 0.1 μCi of ethylene-14C-diquat (Radiochemical Centre, Amersham; specific activity for both approximately 30 mCi mmol -1), and the required concentration of non-radioactive paraguat or diquat dichloride. After incubation, slices were washed by transfer to fresh medium without bipyridyl, blotted and dissolved in 1.0 ml Soluene (Packard Instrument Company Limited). Scintillator [10 ml Dimilume (Packard Instrument Company Limited)] was added and the radioactivity of the slice measured using a liquid scintillation spectrometer. 0.1 ml of media was diluted to 1.0 ml with water and the radioactivity measured after addition of 10 ml Instagel scintillator (Packard Instrument Company Limited).

The amount of diquat found in rat lung slices incubated in media containing diquat (10-5-10-3 M) remained constant from 30 min to 2 h (Fig. 1a). The plateau value obtained was dependent on the concentration present in the medium (Fig 1a). In contrast, paraquat was accumulated linearly from 30 min to 2 h (and in experiments not reported here, for up to 4 h) (Fig. 1b). The amounts of paraquat accumulated were in excess of those seen with diquat. When KCN (10-3 M) plus iodoacetate (10-3 M) were added to the medium either at the beginning of the incubation or after 1 h, this continued

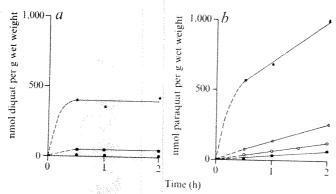


Fig. 1 The accumulation of paraquat and diquat by slices of rat lung. The medium contained the following concentration of bipyridyl:  $\bullet$ ,  $1 \times 10^{-5} \, \text{M}$ ;  $\bigcirc$ ,  $2 \times 10^{-5} \, \text{M}$ ;  $\triangle$ ,  $4 \times 10^{-5} \, \text{M}$ ;  $\blacksquare$ ,  $1 \times 10^{-4} \, \text{M}$ ;  $\triangle$ ,  $1 \times 10^{-3} \, \text{M}$ . Individual points are the mean values from four slices.

accumulation of paraquat was inhibited (Fig. 2a). Identical inhibition has been demonstrated with both 10-5 and 10-4 M paraquat present in the medium. Rotenone (10<sup>-5</sup> M) a specific inhibitor of mitochondrial respiration also inhibits uptake of paraquat (Fig. 2b). Thus, the accumulation of paraquat into slices of rat lung to amounts in excess of those seen with diquat is energy-dependent. A plot of rate of uptake (v) against the rate divided by the concentration of paraquat in the

medium is linear (Fig. 3). From these data, an apparent  $K_{\rm m}$  of  $7 \times 10^{-5}$  M and a  $V_{\rm max}$  of 300 nmol paraquat per g wet

weight per h can be calculated for this process.

After oral administration of 126 mg paraquat per kg body weight to rats, the lung concentration was shown to rise from approximately 4 µg per g wet weight at 4 h to approximately 14 µg per g wet weight at 32 h (ref. 9). During this time, the plasma concentration was constant at around 1 µg ml-1. Paraquat is removed rapidly from the blood of rats after intravenous administration7. The maintenance of a constant concentration of 1 µg ml<sup>-1</sup> for approximately 30 h can, therefore, only be the result of release of paraquat from other organs into the blood, or impaired renal function, or both. Since the gastrointestinal tract of rats was shown to contain a large proportion of the oral dose, this is the most likely source of the blood paraquat. The lung was clearly able to accumulate paraquat to levels in excess of the blood concentration. Similar experiments with diquat given orally to rats show that no such accumulation occurs (L. L. Smith and M. S. Rose, unpublished

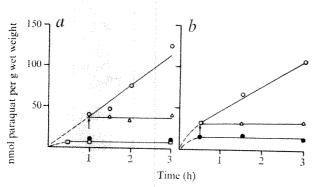


Fig. 2 The effect of metabolic inhibitors on the accumulation Fig. 2 The effect of metabolic innibitors on the accumulation of paraquat by slices of rat lung. a, The medium contained:  $\bigcirc$ , paraquat  $(1 \times 10^{-5} \text{ M})$ ;  $\bigcirc$ , diquat  $(1 \times 10^{-5} \text{ M})$ ;  $\bigcirc$ , paraquat  $(1 \times 10^{-5} \text{ M})$  plus KCN  $(10^{-3} \text{ M})$  plus iodoacetate  $(10^{-3} \text{ M})$ ;  $\triangle$ , paraquat  $(1 \times 10^{-5} \text{ M})$  plus KCN  $(10^{-3} \text{ M})$  plus iodoacetate  $(10^{-3} \text{ M})$  added at arrow. b, The medium contained:  $\bigcirc$ , paraquat  $(1 \times 10^{-5} \text{ M})$ ;  $\bigcirc$ , paraquat  $(1 \times 10^{-5} \text{ M})$  plus rotenone  $(1 \times 10^{-5} \text{ M})$ ;  $\triangle$ , paraquat  $(1 \times 10^{-5} \text{ M})$  plus rotenone  $(1 \times 10^{-5} \text{ M})$  added at the arrow. Individual points are the mean values from two slices are the mean values from two slices.

work). Thus the rat lung accumulates paraquat but not diquat both in vivo and in vitro.

Since it is known that the response of human lung to oral paraquat is delayed, it is possible that a similar accumulation process occurs in man. Therefore, it is of paramount importance that, after ingestion of paraquat, all possible measures are taken to remove paraquat not only from the stomach, but also from the rest of the gastrointestinal tract and blood.

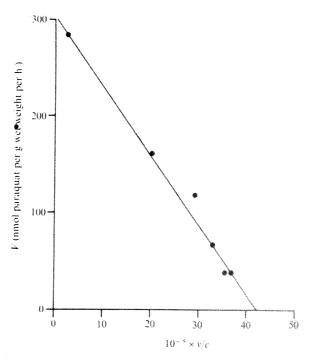


Fig. 3 The derivation of an apparent  $K_m$  and  $V_{max}$  for the uptake process.

Further work is in progress in these laboratories to investigate the mechanism of this uptake of paraquat into the lung. If the process can be inhibited in vivo it may be possible to prevent lung damage.

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### Phytoalexin production by live cells in broad bean leaves infected with Botrytis cinerea

STUDIES on the role of phytoalexins in disease resistance in plants have been limited by the absence of techniques for the accurate localisation of the inhibitors within infected tissues. As a result the postulate of Müller and Börger<sup>1</sup> that phytoalexin production is confined to cells undergoing necrobiosis remains in doubt<sup>2</sup>.

The closely related phytoalexins wyerone and wyerone acid accumulate in leaves of broad bean following fungal infection<sup>3.4</sup>. Although Deverall and Vessey<sup>5</sup> suggested that phytoalexins from *Vicia faba* might be produced by healthy cells, further work by Mansfield and Deverall<sup>6</sup> led them to argue that wyerone acid production was confined to cells undergoing necrobiosis and browning. Here we report the use of fluorescence microspectrography to confirm that wyerone acid is produced by living cells in bean leaves infected with *Botrytis cinerea*.

Leaves were detached from field grown plants of broad bean cv. the Sutton and inoculated on the lower epidermis with 10  $\mu$ l droplets of sterile distilled water each containing about 5,000 conidia of *B. cinerea*, or water alone? Sites inoculated with *B. cinerea* were flecked dark brown after 2 d when droplets were collected and the underlying epidermis peeled off and stored at  $-20^{\circ}$  C. Both wyerone and wyerone acid accumulated in infected epidermal tissues but only the acid was found in inoculum droplets (Table 1). Assuming that wyerone acid in droplets originated in the epidermis the ratio of wyerone acid:wyerone produced by epidermal cells was about 21:1. The phytoalexins could not be detected in leaves inoculated with water alone.

Examination of epidermal strips by fluorescence microscopy revealed that cells that had undergone browning in response to infection by *B. cinerea* absorbed ultraviolet light but many adjacent healthy cells fluoresced blue/green. The vitality of the fluorescing cells was confirmed by their plasmolysis in 2 M sucrose and accumulation of neutral red vital stain. Fluorescence was associated with the cell vacuole. No fluorescent cells were observed in epidermis inoculated with water alone.

Both wyerone and wyerone acid fluoresce under ultraviolet light and these compounds were identified as the possible cause of the fluorescence in infected tissues by microspectrography. Examples of the almost identical spectra obtained from fluorescent cells and solutions of the purified phytoalexins are given in Fig. 1. Similar spectra were obtained for ethanolic solutions of both wyerone and wyerone acid on a standard spectrophotofluorometer (Aminco-Bowman 4–8202SPF). In view of the much greater concentrations of wyerone acid than wyerone

Table 1 Yield of wyerone and wyerone acid from inoculum droplets and underlying epidermis collected from 200 inoculation sites

	Yield (μg)			per g fresh al tissue (µg)
	Wyerone	Wyerone acid		Wyerone acid
Droplet	0	20.0	0	182
Epidermis	2.4	30.5	21.8	277
Total	2.4	50.5	21.8	459
***************************************				

All yields are the mean of results from two experiments. Epidermal tissue (0.1 and 0.12 g) was ground in methanol in a hand homogeniser. The methanol extract was evaporated to dryness in vacuo and partitioned three times between water (5 ml) and diethyl ether (10 ml). Wyerone and wyerone acid were purified from the ether soluble fraction by thin layer chromatography (TLC) on Merck silica gel precoated plates 0.25 mm thick developed in saturated tanks with diethyl ether: methanol (4:1, 8 cm run) then in dichloromethane: petroleum ether  $60-80^{\circ}$  C (2:1, 16 cm run). Wyerone and wyerone acid were detected as bands fluorescing blue under ultraviolet light (wavelength 366 nm) at final  $R_{\ell}$  0.6 and  $R_{\ell}$  0.3, respectively. After elution from silica gel with methanol the concentrations of the phytoalexins were recorded by ultraviolet spectrophotometry<sup>3</sup>. Inoculum droplets (1.1 and 0.9 ml) were partitioned three times with 5 ml diethyl ether and the phytoalexins isolated from the ether phase by TLC as described above.

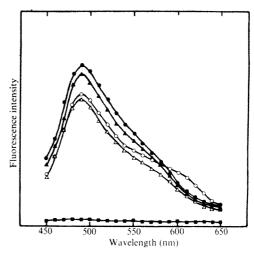


Fig. 1 Fluorescence emission spectra recorded with a Leitz microspectrograph using xenon lamp 405 nm excitation. Spectra recorded for the vacuolar region of different fluorescent cells before (●) and after (○) plasmolysis in epidermal tissue inoculated with B. cinerea and a cell from uninfected epidermis (■). Spectra of wyerone (Δ) and wyerone acid (▲) dried on to microscope slides from solution in ethanol and mounted in distilled water.

extracted from epidermal tissues it is probable that the fluorescence of live cells was largely caused by wyerone acid in the cell vacuole.

The presence of wyerone acid in live cells could result from its production by these cells or accumulation from adjacent necrotic cells and surrounding intercellular fluids. The occurrence of isolated, brightly fluorescent cells, however, rather than a zone of fluorescent tissue around necrotic sites strongly suggested that individual cells were actively synthesising the phytoalexin. The fluorescence of wyerone acid in necrotic cells may have been masked by the accumulation of ultraviolet-absorbing substances during cell browning. We conclude that wyerone acid can be produced by live cells within infected tissue.

These results raise several possibilities concerning the relationship between host cell death and the production of wyerone acid by the broad bean plant. Phytoalexin production may be triggered initially by cell death in response to fungal invasion and metabolites released from dead cells may induce subsequent biosynthesis of wyerone acid in adjacent living cells. Alternatively fungal metabolites may act as specific inducers of phytoalexin production which may be confined to live cells where wyerone acid may ultimately reach phytotoxic concentrations and cause host cell death. It is hoped that further use of the microspectrograph and detailed microscopical observations on the early stages of infection will allow these possibilities to be examined.

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### Data processing by the chemotaxis machinery of Escherichia coli

THE chemotactic behaviour of Escherichia coli provides a useful model for study of the molecular basis of a simple stimulusresponse sequence. Chemical stimuli are detected by specific receptors2 which in turn alter the rotation of the flagella3 to elicit movement towards attractants or away from repellents. About twenty types of chemoreceptors have been identified in E. coli<sup>4-6</sup>, suggesting that a communication system transmits sensory data from the receptors to the flagella, and that signals from different receptors converge either before or on reaching the flagella. I have attempted to dissect this system by isolating and examining nonchemotactic (che) mutants of the type first described in E. coli by Armstrong et al.7. In this report I summarise the properties of che mutants and derive a model of the chemotaxis machinery based on this genetic analysis.

Chemotaxis in E. coli is mediated through variations in the frequency of directional changes (tumbles) that occur during swimming8.9. When bacteria sense an increase in attractant concentration8-10, or a decrease in repellent concentration11, tumbling is suppressed, causing net migration in the desired direction. Berg and Brown<sup>8</sup> proposed that tumbles are initiated by a 'tumble generator' and that tumble frequency is controlled by chemoreceptor signals that modulate this generator. Since E. coli swim by rotating their flagellar filaments<sup>12,13</sup>. tumbling depends on the direction of rotation3. Counterclockwise rotation causes straight swimming; reversal to a clockwise direction initiates tumbling.

Chemotaxis mutants that are motile but cannot respond to any stimuli (che mutants) should define common components of the chemotaxis machinery, including the tumble generator, provided that such components are not an essential part of the flagellum or its motor. Table 1 summarises the swimming behaviour of the che mutants studied by Armstrong et al.7,14 and myself (in preparation).

All the mutants, which represent four genes, exhibit aberrant swimming patterns due to incessant tumbling or loss of tumbling. This result indicates that most or all of the common components of the chemotaxis machinery that are not essential for viability are required for generating and regulating tumbles.

Table 1 Properties of generally nonchemotactic (che) mutants

Complementa-	No. of independer	it isolates	Swimming
tion Class*	Armstrong et al.7.14	Parkinson <sup>†</sup>	pattern!
che A	16	72	No tumbling
cheB (type 1)	21	68	No tumbling
cheB (type 2)	en titologico de la constanta	26	Incessant tumbling
cheC	1	3	No tumbling
che D	Manageries -	3	No tumbling
CHE D	warmhara.	3	No tumbling

<sup>\*</sup>In the original complementation study of Armstrong and Adler14, gene assignments were made on the basis of abortive transduction tests. These assignments were confirmed† using F-prime episomes to construct stable partial diploids.

†This work is in preparation. ‡Bacteria were grown at 35° C in tryptone broth to an absorbance at 590 nm of 0.3, and were either observed directly with the light microscope or were first washed and resuspended in 10 mM potassium phosphate (pH 7) plus 0.1 mM EDTA. Swimming patterns are the same in either case.

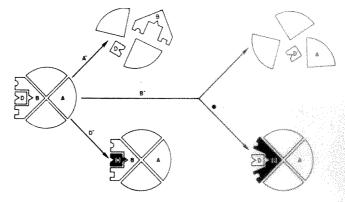


Fig. 1 A model of the tumble generator based on the properties of generally non-chemotactic (che) mutants. A further explanation is given in the text.

Although mutations in the che genes can lead to altered tumbling patterns, this does not necessarily mean that the product of each gene is required directly for tumbling. Che mutants might define not only parts of the tumble generator but also components that interact with the generator to modify tumbling behaviour. The role of each che gene product can best be inferred from mutants that lack entirely the product or function of a che gene. As the null phenotypes of the che genes reveal (Table 2), several che gene products are not involved directly in generating tumbles. The product of the cheC gene seems to be an essential component of the flagellum since Silverman and Simon<sup>15</sup> showed that in its absence the flagellum is not assembled. Motile, nonchemotactic cheC mutants cannot tumble, and must represent special alterations of the cheC product that permit assembly of a flagellum that can rotate only counter-clockwise.

The product of the cheD gene is not required for tumbling, since in its absence, tumbling is normal although chemotaxis to serine5.16 and some repellents6 is lost. But, some alterations of the cheD product do lead to a loss of tumbling ability. Since these mutants are invariably dominant (my work in preparation), it seems that an appropriately altered cheD product can interact with and inhibit the tumble generator.

Table 2 Phenotypes of null mutants in the che genes

Gene che A che B che C che D	Swimming phenotype No tumbling No tumbling Nonmotile Wild type <sup>8</sup>	Chemotaxis phenotype Generally non-chemotactic Generally non-chemotactic Non-chemotactic (no flagella) <sup>15</sup> Specifically non-chemotactic to serin	ie <sup>a, 16</sup>
che D	Wild type <sup>8</sup>	Specifically non-chemotactic to ser and to some repellents <sup>6</sup>	

Null mutants are those with a complete loss or with total inactivity of a particular gene product. The null phenotypes of the che genes were determined in various ways, most of which involve the study of nonsense or deletion mutants in these genes (my work in preparation).

Thus the normal cheD product may function as a 'switch' to control the tumble generator in response to serine signals and some repellent signals. In nonchemotactic cheD mutants this switch may be frozen in the 'off' position to prevent tumbling.

The products of the cheA and cheB genes are probably part of the tumble generator since null mutations in these genes result in a nontumbling phenotype (Table 2). The cheB product, however, seems to have a dual role which is reflected in the two tumbling patterns of cheB mutants (Table 1). The non-tumbling cheB mutants indicate that cheB product is needed to generate tumbles, and the incessantly tumbling cheB mutants indicate that cheB product also plays a part in regulating tumble frequency.

What is the nature of the defect in the incessantly tumbling (uncoordinated17) cheB mutants? On the one hand, tumbling mutants might have hyperactive tumble generators, and if this is the case, each mutant should respond equally well or poorly

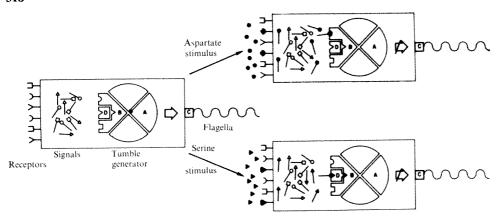


Fig. 2 The role of the tumble generator in chemotaxis. A further explanation is given in the text.

to different stimuli. On the other hand, tumbling mutants might be poorly coupled to the receptor signals that control tumbling rates, as Berg and Brown suggested. In this case, the tumbling frequency of the mutants represents not an overactive generator but rather a free running generator, unmodulated by receptor signals. If the tumble generator in these mutants is defective in its reponse to modulating signals, different signals might be perceived with different efficiencies and thereby elicit different responses.

Tumbling mutants respond to sudden changes in attractant concentration although not as well as wild type (Table 3 and refs 3 and 18). In most cases tumbling mutants do not respond with the same efficiency to the attractants aspartate and serine which are detected by different chemoreceptors. Some mutants (e13p1, e16f1, e19e1, e27f1) respond to serine but respond poorly or not at all to aspartate; other mutants (e16h1, e16n2, e17a1, e20t2, e26e2, e28h2) respond to both types of stimuli,

Table 3 Swimming behaviour of tumbling mutants in response to temporal stimulation with the attractants aspartate or serine

Duration (s)	of response		
Aspa	rtate	Ser	rine
3-fold jump	30-fold jump	3-fold jump	30-fold jump
$101 \pm 12 (7)$	$122\pm10(3)$	$346 \pm 38 (4)$	> 600 (3)
None (3)	None (5)	$98\pm 7(3)$	$100 \pm 13 (5)$
None (3)	None (5)	$65 \pm 10 (5)$	$89 \pm 6 (3)$
$62 \pm 8 (5)$	88 ± 5 (2)	$121 \pm 16 (5)$	$188 \pm 10 (2)$
$79\pm13(5)$	$112 \pm 7(2)$	$167 \pm 28 (5)$	$243 \pm 12 (2)$
84± 9 (5)	$111 \pm 8 (2)$	$94\pm 7(5)$	$144 \pm 11 (2)$
None (3)	$50 \pm 7 (5)$	$64 \pm 12 (5)$	$107 \pm 11 (3)$
$67\pm12(5)$	$109\pm13(2)$	$140\pm12(5)$	$187 \pm 11$ (2)
None (3)	None (3)	None (3)	$66\pm11(5)$
$43 \pm 5 (5)$	$96\pm18(3)$	$74 \pm 9 (5)$	$107 \pm 4(2)$
None (5)	61 + 4(2)	$99 \pm 17 (5)$	$154 \pm 13$ (2)
$63\pm 7(5)$	$99\pm10(2)$	$111\pm12(5)$	183 (1)
	Aspa 3-fold jump $101\pm12$ (7) None (3) None (3) $62\pm8$ (5) $79\pm13$ (5) $84\pm9$ (5) None (3) $67\pm12$ (5) None (3) $43\pm5$ (5) None (5)	Aspartate 3-fold jump 30-fold jump $101\pm12$ (7) $122\pm10$ (3) None (3) None (5) None (3) None (5) $62\pm8$ (5) $88\pm5$ (2) $79\pm13$ (5) $112\pm7$ (2) $84\pm9$ (5) $111\pm8$ (2) None (3) $50\pm7$ (5) $67\pm12$ (5) $109\pm13$ (2) None (3) None (3) $43\pm5$ (5) $96\pm18$ (3) None (5) $61\pm4$ (2)	3-fold jump 30-fold jump 101 $\pm$ 12 (7) 122 $\pm$ 10 (3) 346 $\pm$ 38 (4) None (3) None (5) 98 $\pm$ 7 (3) None (3) None (5) 65 $\pm$ 10 (5) 62 $\pm$ 8 (5) 88 $\pm$ 5 (2) 121 $\pm$ 16 (5) 79 $\pm$ 13 (5) 112 $\pm$ 7 (2) 167 $\pm$ 28 (5) 84 $\pm$ 9 (5) 111 $\pm$ 8 (2) 94 $\pm$ 7 (5) None (3) 50 $\pm$ 7 (5) 64 $\pm$ 12 (5) 109 $\pm$ 13 (2) 140 $\pm$ 12 (5) None (3) None (3) None (3) None (3) None (3) None (5) 61 $\pm$ 4 (2) 99 $\pm$ 17 (5) None (5) 61 $\pm$ 4 (2) 99 $\pm$ 17 (5)

\*Bacteria were grown at 35° C in minimal salts-glycerol medium to an optical density at 590 nm of 0.2. The cells were washed and resuspended in 10 mM potassium phosphate (pH7) containing 0.1 mM EDTA at an optical density at 590 nm of 0.1. Samples of the bacteria were then incubated at room temperature for 15 min in the presence of 0.1 mM attractant (either aspartate or serine) before an experiment began. To minimise changes in background attractant concentrations, each set of measurements was completed within 15 min thereafter. Drops containing 20 µl of bacteria in attractant were placed on microscope slides and incubated for 1 min. The cells were then stimulated by the addition of 1 µl of attractant, bringing the final concentration to 0.3 mM (3-fold jump) or to 3 mM (30-fold jump). If the bacteria could respond, they stopped tumbling and began to swim in straight lines for a period that varied directly with the stimulus size. The times shown are subjective estimates of the time it took for 50% of the responding cells to begin tumbling once again. Differences between independent observers were not significant and the data were pooled to give the averages and standard deviations and (in parentheses) the number of measurements made. These estimates are quite accurate for tumbling mutants because the two behavioursswimming and tumbling—are very distinct and the transition time is short relative to the response time. The wild type is more difficult to measure because its normal tumble frequency is not as great as in tumbling mutants.

†Each of these tumbling mutations is in the cheB gene. All the mutant alleles were transduced into a common genetic background for these experiments. Mutants e20t2, e26e2, and e27f1 are amber (nonsense) mutants.

and one (e21e1) responds poorly to both. Comparisons between mutants (for example, e17a1 with e27f1 or e16n2 with e28h2) show that two mutants can have the same response to one stimulus but a different response to another stimulus. These results suggest that tumbling mutants cannot be modulated properly by chemoreceptor signals and that these signals control tumbling frequency by interacting with the *cheB* product.

A model of the tumble generator based on these findings is presented in Fig. 1. The generator has three components (and perhaps others not yet detected by mutation) each of which can mutate to produce a generally nonchemotactic phenotype in one or more ways. The cheC product is assumed to be a component of the flagellum rather than a part of the tumble generator itself. The products of the cheA and cheB genes are essential components of the tumble generator. Both are required to generate tumbles, but the cheB product also controls tumbling rate by interacting with signals from the chemoreceptors. The cheD product is also thought to be a component of the tumble generator although the cheD product is not required for tumbling. Rather, the cheD product is a component of the tumble control system, passing serine signals and some repellent signals to the tumble generator.

Since the cheB product is ultimately responsible for controlling tumbling rate in response to chemoreceptor signals, the model assumes that the cheD product interacts directly with the cheB component in transmitting signals to the tumble generator. This interaction can be demonstrated in three ways. First, it is possible to isolate suppressors of cheD mutants that are mutations in the cheB gene. Second, double mutants containing a cheB tumbling mutation and a cheD nontumbling mutation tumble either incessantly or not at all depending on the severity of the cheB defect as measured by temporal stimulation. Third, the response of tumbling cheB mutants to aspartate stimuli can be altered greatly by cheD null mutations, suggesting that the configuration of the cheB product can be changed by removing the cheD product from the generator. (A complete description of experiments on the interaction of the cheB and cheD products will be presented separately.)

The postulated role of the tumble generator in chemotaxis is shown in Fig. 2. The tumble generator is probably controlled in a negative manner by inhibitory signals whose level is modulated by the various chemoreceptors. In the absence of stimuli, the level of inhibitory signals is low, and consequently tumble impulses are frequently sent to the flagella. The level of inhibitory signal rises whenever stimuli are detected by the chemoreceptors; different receptors control different inhibitory signals. Aspartate signals (and presumably several others) interact directly with the *cheB* component, whereas serine signals and some repellent signals are transmitted to the tumble generator through the *cheD* product.

When E. coli are presented simultaneously with an attractant and a repellent the response depends on the relative strengths of the conflicting stimuli<sup>11,19</sup>. The model of the chemotactic process presented in Fig. 2 implies that opposing stimuli can be integrated at several points depending on the nature of the

stimuli. For example, the chemoreceptors for serine and for some repellents may control the same signal, in which case integration would take place at the signal level. If different signals are controlled by these receptors, they would be integrated at the cheD product. Other combinations of stimuli. for example, aspartate and a repellent, would be integrated by the cheB product.

These studies of chemotaxis mutants indicate that at least two types of chemoreceptor signal are transmitted to the tumble generator. Although nothing is known about these signals, it seems unlikely that they will prove to be electrical unless each signal is associated with a different class of ions. Perhaps chemoreceptors communicate with the tumble generator by mechanical or chemical means. In any event, the central role of these signals in the chemotactic process emphasizes the importance of learning more about their nature and their mode of transmission.

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#### Transfection of non-host bacterial spheroplasts with bacteriophage ФX174 DNA

BACTERIOPHAGE ФX174 containing circular single-stranded DNA (SS-DNA) can infect Escherichia coli C and Shigella<sup>1-4</sup>. Progeny phages are produced through synthesis of circular double-stranded DNA (replicative form, RF-DNA) as the intermediate5,6. ΦX174 cannot infect bacteria which have no specific receptors for phage adsorption on the cell wall. The host range in  $\Phi X$  infection is thought to be determined primarily by success or failure of adsorption and if most of the cell wall is removed from the bacterium, the host range is extended and  $\Phi X$ -DNA can infect E. coli strains other than the host, and produce progeny phages. But if the recipient bacterial spheroplasts are of species distantly related to the host,

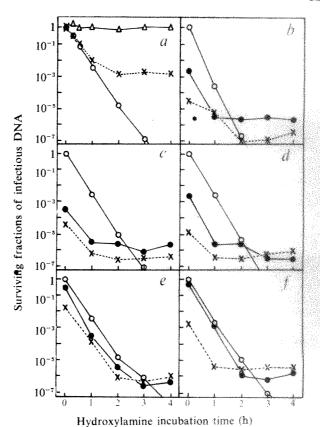


Fig. 1 Hydroxylamine treatment of the infectious materials extracted from  $\Phi X$ -SS-DNA-transfected spheroplasts. The bacterial spheroplasts ( $10^9$  cells ml<sup>-1</sup>) were incubated with SS-DNA ( $10^{12}$  molecules ml<sup>-1</sup>) for 15 min and then PAM medium was added and incubation was continued further (see legend of Table 1). At intervals, spheroplasts were lysed by the addition of 1/20 volume of 12.5% Sarkosyl NL97 and the lysates were extracted by phenol and nucleic acids were precipitated by alcohol, resuspended in 0.05 M Tris-HCl, pH 6.0, added with 1/5 volume of 1 M hydroxylamine-HCl, pH 6.0, and incubated at 37° C. At intervals, aliquots were diluted and their surviving infectivity was assayed on E. coli K12W6 spheroplast. The surviving fraction was expressed as the ratio of PFU in spheroplast assay at the indicated time of hydroxylamine treatment to PFU of the sample not treated with hydroxylamine in which SS-DNA was added to spheroplasts previously lysed with Sarkosyl and phenol. •, 15 min incubation before the addition of PAM medium; ×, 15 min incubation after the addition of PAM medium; O, SS-DNA added to the previously lysed spheroplasts. a, E. coli K12W6, Purified SS-DNA (O), RF-DNA (A) and SS-, RF-DNA mixture (×), incubated with 0.2 M hydroxyla-mine-HCl, pH 6.0, at 37° C. b, K. pneumoniae; c, S. marcescens; d, P. vulgaris; e, P. aeruginosa; f, E. coli K12W6.

ΦX-DNA may not be able to penetrate into the spheroplasts and replicate and produce progeny phages as well as in the host spheroplast. It is known that  $\Phi X$  phage replication in E. coli depends largely on host functions; for example, the ΦX-DNA replication is greatly affected by several genes responsible for host DNA synthesis<sup>7,8</sup>. On this basis, ΦX-DNA replication in bacteria distantly related to the natural host might on the one hand fail because of incompatibilities between  $\Phi X$ -DNA and bacterial gene functions or on the other hand be successful because common gene functions are widespread throughout a wide range of bacterial species. We tried to transfect OX-DNA to various bacteria belonging to the Enterobacteriaceae and Pseudomonas aeruginosa to examine the capability for phage production in bacteria other than the host, and found that ΦX-RF-DNA synthesis proceeded as efficiently as in the natural host in distantly related species of bacteria, but that progeny phage production was greatly reduced. Bacterial relationships determined on the basis of DNA homology.

Preparation of spheroplasts and transfection were performed

Table 1 Efficiency of transfection in various bacterial spheroplasts

	Effi	ciency	
Bacterium	I,	nput	DNA
	SS-DNA	RF-DNA	homology10
Escherichia coli K12W6	$4.1 \times 10^{-3}$	$8.0 \times 10^{-5}$	101%
В _	$5.3 \times 10^{-4}$	$2.4 \times 10^{-6}$	100
$\widetilde{\mathbf{C}}^{\mathbf{r}\mathbf{\Phi}}$	$2.1 \times 10^{-4}$	$3.0 \times 10^{-6}$	-punctions.
Salmonella typhimurium	$2.1 \times 10^{-6}$	$2.9 \times 10^{-8}$	71
Aerobacter aerogenes	$1.4 \times 10^{-8}$	$3.5 \times 10^{-9}$	51
Klebsiella pneumoniae	$4.5 \times 10^{-8}$	$1 \times 10^{-10} >$	25
Proteus vulgaris	1.0×10 <sup>-9*</sup>	$1 \times 10^{-10} >$	14
Serratia marcescens	$2.1 \times 10^{-8}$	$1 \times 10^{-10} >$	7
Pseudomonas aeruginosa	$1.5 \times 10^{-9*}$	$1 \times 10^{-10} >$	1

Spheroplasts of all bacterial strains were prepared essentially according to the method of Guthrie and Sinsheimer. DXam3-SS-DNA (1010 molecules ml<sup>-1</sup>) in 0.05 M Tris-HCl, pH 8.1, was added to an equal volume of spheroplast stock solution in PA medium (10° spheroplasts ml<sup>-1</sup>) and incubated for 15 min at 37° C. After an addition of eight volumes of PAM medium, incubation was continued for 120 min and then the spheroplasts were lysed with chloroform and the titre of the lysates was determined. Transfection of  $\Phi$ Xam3-RF-DNA (10<sup>10</sup> molecules ml<sup>-1</sup>) was performed in the same manner as SS-DNA. The efficiency of transfection which is the average of eight experiments is expressed as plaque forming units (PFU) obtained per input  $\Phi X$ -DNA molecule. Data of DNA homology are cited from McCarthy and Bolton<sup>10</sup>

\*Values indicate averages of several experiments where plaque formation was observed.

essentially by the method of Guthrie and Sinsheimer9. The percentage of bacteria surviving after the osmotic burst treatment was less than 0.5% in all spheroplast preparations. In the transfection of E. coli K12, B and C<sup>r</sup>Φ (a ΦX-resistant mutant of E. coli C) the number of progeny phages produced was proportional to the amount of input  $\Phi X$ -DNA until saturation occurred at about 10³ ΦX-SS-DNA molecules per spheroplast. The efficiencies of transfection of  $\Phi X$ -SS-DNA and RF-DNA are shown in Table 1, together with the homology of the bacterial DNA reported by McCarthy and Bolton<sup>10</sup>.

In the case of SS-DNA transfection with Proteus vulgaris and P. aeruginosa spheroplasts, phage production was sometimes not observed, probably as a result of inefficient and variable competence in transfection. The efficiency of RF-DNA transfection was lower than that of SS-DNA transfection in E. coli K12W6 (ref. 11) and about 50 times lower than our own results with SS-DNA. For instance, progeny phages could not be found in RF-DNA transfection in Klebsiella pneumoniae, P. vulgaris, Serratia marcescens and P. aeruginosa, probably as a result of phage production at a level too low to be detected.

We therefore examined whether  $\Phi X$ -RF-DNA synthesis occurred in spheroplasts of these bacteria. To determine RF-DNA in the presence of excess input SS-DNA, DNA was extracted by phenol from spheroplasts infected with SS-DNA at intervals and treated with hydroxylamine which inactivates SS-DNA but not RF-DNA<sup>12</sup>. The amount of RF-DNA synthesised was determined from the surviving, hydroxylamineresistant infectivities in a spheroplast assay on E. coli K12W6.

Table 2 Efficiencies of RF synthesis and phage production

	Effic	ciency
Bacterium	RF equivalent per spheroplast	Phage per RF equivalent
E. coli K12W6	$2.3 \times 10^{-2}$	2.0×10
K. pneumoniae	$2.3 \times 10^{-3}$	$1.3 \times 10^{-5}$
S. marcescens	$7.7 \times 10^{-3}$	$3.2 \times 10^{-6}$
P. vulgaris	$1.0 \times 10^{-2}$	$2.4 \times 10^{-7}$
P. aeruginosa	$2.3 \times 10^{-2}$	$4.3 \times 10^{-7}$

Mean value of PFU in the spheroplast assay 3 and 4 h after the addition of hydroxylamine in Fig. 1b-f was taken as infectivity of RF-DNA. The number of RF molecules was calculated from the standard curve (not shown) of phage-yield against RF-DNA molecule in the spheroplast assay.

As shown in Fig. 1a, the infectivity of SS-DNA decreased to less than 10-7 after hydroxylamine treatment for 3 h, whereas that of RF-DNA was not affected. In all bacteria tested it was found that the infectivity surviving after hydroxylamine treatment for 4 h was almost as much as that of E. coli K12W6 (Fig. 1b-f). Decreased infectivity of input SS-DNA (see time zero of hydroxylamine treatment) was found in most bacteria tested, especially in P. vulgaris, S. marcescens and K. pneumoniae and is probably the result of the action of extracellular and intracellular DNases. Infectivity decreased as the time of incubation of DNA with spheroplast was increased. The frequency of hydroxylamine resistant infectious materials together with that of phage production is shown in Table 2. It seems that synthesis of RF-DNA (at least parental RF) proceeded to a similar degree in all bacteria, irrespective of phage production. Therefore, variable capability to produce progeny phage seems to be caused by a difference in blocking of later processes.

There seems to be some correlation between the efficiency of phage production and the order of bacterial DNA homology. This is not a simple reflection of difficulty of DNA penetration into the bacterial cell or degradation of  $\Phi X$ -DNA by DNases, if any, before and/or after penetration, since apparently there were no great differences in the extent of RF-DNA synthesis among all the bacteria tested. It has been reported that T4 phage treated with urea transfects spheroplasts of Salmonella anatum, P. vulgaris, S. marcescens and Aerobacter aerogenes with a higher efficiency of progeny phage production13 than  $\Phi X$ -DNA. The difference of efficiency between 14 and  $\Phi X$  might reflect differences in dependence of phage growth on host functions. Phage T4 has a complex genetic composition which makes it highly autonomous compared with ΦX which is less complex and needs several host gene functions even for the conversion of SS-DNA to parental RF14.15. There seem to be common factors in the machinery of RF synthesis in the bacteria tested, although the more distant is the relationship with the host, the less affinity or combatibility is there between the host functions and phage products with a concomitant reduction in phage production. The characterisation of hydroxylamine-resistant infectious material is now in progress, as is the examination of restriction as one of the possible mechanisms for a low efficiency of phage production in some bacteria.

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#### Selective blockage of bacteriophage T<sub>4</sub> assembly by chemical modification

A QUESTION of fundamental importance to structural biology is the molecular nature of the contact points which direct the protein assembly process in an extraordinarily selective and efficient manner. This problem is closely parallel to other areas of biology involving macromolecular recognition, such as enzyme/substrate specificity and antigen-antibody interaction. A promising system for the study of such structural problems is the bacteriophage T4 which is a macromolecular in the process of uniting the half fibres and attaching the fibres to the otherwise complete phage particle. The basic strategy employed was to subject a defective lysate to a specific modification reaction and then see if the lysate loses the ability to donate the specified phage component, in a suitable in vitro complementation test, while the background, completely assembled particles, survive unscathed. In this way attention was focused on critical sites exposed only in the unassembled state. To further focus on those sites which may be involved in the attachment process (as opposed to, for example, interferring with the infection process), a post-activation procedure was instituted whereby it could be ascertained not only that a modified site had blocked assembly but, furthermore, that the modified component had not been assembled to form a non-infectious particle. While hoping to study the actual contact sites, we recognise that an inherent weakness of this kind of approach is the a priori inability to distinguish modification at an 'active centre' from modification at a distant site which effects the observed blockage through some sort of unspecifiable conformational mechanism.

	vitro complementation between fibre defective lysates

Treatment	Reference	pΗ	Expected target	Result
Maleic anhydride	9	7.0	Amino	Complementation reflected sensitivity of whole phage
1-fluoro-2,4-dinitrobenzene (FDNB)	10	9.0	Sulphhydryl and amino	Complementation reflected sensitivity of whole phage
N-bromosuccinimide (NBS)	11	7.0	Sulphhydryl and aromatic	Complementation reflected sensitivity of whole phage
N-ethylmaleimide (NEM)	9	7.0	Sulphhydryl and amino	Complementation reflected sensitivity of whole phage
Woodward's reagent K	12	7.0	Carboxyl	Complementation reflected sensitivity of whole phage
Rose Bengal photoxidation	13	7.8	Imidazole	Whole phage and complementation insensitive
Iodoacetate, iodopropionate, iodoacetamide	14	5.0	Thioether	Whole phage and complementation insensitive
Iodoacetamide Iodoacetate, iodopropionate, iodoacetamide	14	7.0	Sulphhydryl, imidazole, and thioether	Complementation impaired while control phage insensitive

For each reagent tested, defective lysates lacking proximal half fibres (34-:34am A 455), distal half fibres (37-:37am N91) or whole fibreless particles (23-/10-:23am H11/10am B255) were prepared as previously described 15. The standard phosphate buffer used throughout this investigation was made of 7 g Na<sub>2</sub>HPO<sub>4</sub>, 3 g KH<sub>2</sub>PO<sub>4</sub>, 4 g NaCl and 1 l H<sub>2</sub>O). After autoclaving, 10 ml of 0.1 M MgSO<sub>4</sub> was added. Before treatment, the lysates were transferred into the appropriate buffers by dialysis in the cold (4° C). The reaction was stopped and the reagent removed by exhaustive dialysis in the cold. The treatment of the control and experimental complex was identical expert for the emission of the modification. exhaustive dialysis in the cold. The treatment of the control and experimental samples was identical except for the omission of the modification reagent. Exposure to the treatment buffers did not inactivate phage or interfere with subsequent complementation. A full description of the treatments may be found in the references cited in the Table. For each treated sample the titre of background phage was measured to determine the effect of modification upon phage viability. *In vitro* complementations were carried out by mixing 25 µl of the treated 34<sup>-</sup>, 37<sup>-</sup> or 23<sup>-</sup>/10<sup>-</sup> defective lysate respectively with 25 µl of (37<sup>-</sup> or 23<sup>-</sup>/10<sup>-</sup>), (34<sup>-</sup> or 23<sup>-</sup>/10<sup>-</sup>) and (37<sup>-</sup>) and incubated for 24 h at 30° C. The mixtures were then diluted with 25 ml of the standard buffer and assayed. Each complementation test of a treated lysate was accompanied by the parallel complementation of the control.

complex built up of discrete assemblies such as head, tail and tailfibres

During the restrictive growth of conditional lethal mutants of this bacteriophage1, phage development is blocked at the step which requires the product of the mutant gene and frequently recognisable structural components accumulate. With the demonstration that many of these components are normal phage precursors which can be used in in vitro studies of virus assembly2, the way is open for direct biochemical investigation of the molecular basis of contact point specificities. From among the many described steps of phage assembly3, the junction of the half fibres and the attachment of the complete tail fibres to the base plate were chosen for closer scrutiny because the molecular composition of both fibre4 and base plate have been thoroughly examined, the contact points are topologically relatively restricted, and the reactions go well in vitro2. The process of fibre attachment seems to pass through an intermediate in which fibres (as half or whole fibres) are transiently bound to head-related 'jig' sites6-8 before final binding to the base plate.

We have investigated the use of specific amino acid modification as a probe for identifying and labelling the sites involved

Various treatments employed in this study and their qualitative outcomes are summarised in Table 1. The results suggest that the assembled phage is sensitive to modification of amino, carboxyl and aromatic side groups while neither assembled phage nor fibre contact sites are sensitive to reagents specific for the thioether or imidazole side groups. Sulphydryl carboxymethylation, however, with the alkylating reagents iodoacetate, iodopropionate and iodoacetamide manifest the requisite selectivity for the assembly process.

Post activation (Table 2) shows that the observed selective impairment of the in vitro complementation is indeed due to blockage of assembly. A series of experiments was carried out in which the experimental conditions (pH, reagent concentration, and duration of treatment) were varied. The results indicate that optimal discrimination between assembly blockage and phage survival is obtained by treating with 100 mM iodoacetate at pH 7.0 for 60 min at 25° C. That the three alkylating reagents used have identical effects, suggests that modification is not very sensitive to either the charge or size of the adduct. Neither the kinetics of modification (as measured by reduction in complementability) nor the kinetics of complementation with modified components, has been exhausively

Table 2 The effect of carboxymethylation upon free fibre components

	Lad	ie 2 The effect of carr	oxymethylation	upon tree nore	components		
Treatment	Mutant	Component	Control (b	ackground)	Complei	mented with:	
		donated	Titre	Percent	Mutant	Titre	Percent
Unmodified		Proximal	$5.9 \times 10^{5}$	100		$2.0 \times 10^{8}$	100
	37-/23-/10-	half			34-		
Modified		fibre	$5.2 \times 10^5$	89		$2.0 \times 10^8$	100
					34 -	background tit	$re = 7.6 \times 10^6$
Unmodified	•	Distal	$2.8 \times 10^7$	100		$2.8 \times 10^{9}$	
	34-/23-/10-	half			37-		
Modified	, ,	fibre	$2.1 \times 10^7$	75		$1.0 \times 10^{8}$	5
					37-	background tit	$re = 6.0 \times 10^6$
Unmodified		Whole	$3.1 \times 10^{5}$	100		$1.4 \times 10^{9}$	100
	23 -/10 -	fibre			37~		
Modified	•		$4.3 \times 10^{5}$	138		$1.0 \times 10^{7}$	1
					37-	background tit	$re = 2.2 \times 10^6$

Defective lysate preparation, modification and complementations were carried out as described in the legend for Table 1. As well as the previously described mutants, the following were used:  $(37^-/23^-/10^-:37am\ N91/23am\ H11/10am\ B255)$  in which the assembly of distal half fibres, heads and tails are blocked and  $(34^-/23^-/10^-:34am\ A455/23am\ H11/10am\ B255)$  in which assembly of proximal half fibres, head and tail is blocked. Titres are expressed as phage ml<sup>-1</sup>. The left hand portion of the Table pertains to the lysates subjected to carboxymethylation. A description of each stock is followed by the titres of the background phage in the treated and parallel untreated aliquots. Percent is a normalised measure of the behaviour of a treated sample relative to the parallel untreated control aliquot. The right hand portion of the Table deals with the complementation of the indicated (untreated) defective lysates with the modified and corresponding unmodified lysates. The results of the complementations are shown both as titres of the diluted complementation mixes and as percent, which is the normalised measure of the behaviour of the modified samples with respect to their unmodified control aliquots. For postactivation, duplicate pairs of complementations involving the modified lysates were set up. After the normal 24 h incubation period, one of the pair was titrated as described and the results were as shown in this Table. To the other of the pair, an aliquot of the corresponding unmodified lysate was added and the mix was incubated a further 24 h before titration. In each case, this post activation yielded 100% phage, indicating that the original incubation with modified components did not irreversibly use up the complementary components in producing inactive phage particles.

examined. Accordingly, we are not in a position to conclude whether or not the modification is a single hit or a multiple hit phenomenon, nor whether the blockage is absolute or simply slows the assembly process.

By a suitable choice of defective lysates subjected to modification, and selection of appropriate defective lysates for *in vitro* complementation, several different subassemblies and their respective contact and 'jig' sites may be differentiated. Table 2 gives the results of experiments which examine the sensitivity to modification of the proximal half fibre, distal half fibre and the complete fibre before attachment to the base plate of the otherwise complete phage particles. Although these experiments were repeated several times, with qualitatively identical results, the details of only one set are reported here.

The striking observation to emerge from these data is that the proximal half fibre, in the absence of other fibre components seems to be insensitive to modification, while both the distal half fibre and the whole fibre are sensitive to such treatment. As the whole fibre and the distal half fibre do not have obvious, exposed assembly contact points in common, the area on the distal half fibre, which recognises the 'distal jig site', is implicated. To explore this possibility further, modification of the half fibres was done in the presence of fibreless particles. An unambiguous interpretation of the results is impossible due to the complexity of the appropriate treatment and complementation mixtures. They are consistent, however, with the notion

that the distal half fibre, when attached to the 'distal jig site', is afforded a degree of protection against modification.

In a complementary series of experiments, the modification of fibreless particles in the presence and absence of unattached half-fibres was examined and a representative set of results is presented in Table 3. It can be seen that the fibreless particles are fairly sensitive to inactivation except that a degree of protection is afforded by the proximal half fibre. This observation suggests an interaction between the proximal half fibre and the fibreless particle, with the particle half of the recognition site being susceptible to modification by alkylating reagents. Recent work (B. E. Terzaghi and E.T., unpublished) with some new mutants (fibre attachment defective) is most readily interpreted in terms of just such a distinct 'proximal jig site' transiently occupied by the proximal half fibre.

While recognising the intrinsic limitations of modification experiments, as well as the very limited amount of quantitative (kinetic) work done, the qualitative results reported here indicate that this may be a fruitful avenue of investigation. By virtue of a differential sensitivity to alkylation at pH 7.0, we have been able to confirm the existence of a distal half fibre site which recognises an assembly 'jig', as well as identifying a site on the fibreless particle which possibly serves as a 'jig' for the proximal half fibre. Thus, a handle has been found for further characterisation of a transient but possibly important type of assembly contact point.

	donated	rger.			nented with:	
		Titre $1.5 \times 10^7$	Percent 100	Mutant	$\begin{array}{c} \text{Titre} \\ 3.1 \times 10^8 \end{array}$	Percent 100
X4E	Fibreless particle	$1.8 \times 10^7$	120	23-/10-	$5.9 \times 10^7$	19
	Fibreless particle,	$1.1 \times 10^6$	100		background titr 2.1 × 10 <sup>9</sup>	$e = 2.0 \times 10^5$
37-	proximal half fibre	$8.8 \times 10^{5}$	80	,	1.4×10°	66
	Fibreless particle,	4.0×10 <sup>6</sup>	100	•	background titr 4.0×10°	$e = 1.0 \times 10^6$
34-	distal half fibre	3.7×10 <sup>6</sup>	92	23 -/10 -	$6.8 \times 10^{8}$	15
	37 <sup>-</sup>	37 proximal half fibre  Fibreless particle,	37- Fibreless particle, proximal half fibre $ \frac{1.1 \times 10^6}{8.8 \times 10^5} $ 34- Fibreless particle, $\frac{4.0 \times 10^6}{4.0 \times 10^6} $	Fibreless particle, proximal half fibre $ \begin{array}{c ccccc}  & 1.1 \times 10^6 & 100 \\ \hline  & 8.8 \times 10^5 & 80 \\ \hline  & Fibreless particle, distal half fibre & 4.0 \times 10^6 & 100 \\ \hline \end{array} $	37- Fibreless particle, proximal half fibre $ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Fibreless particle, proximal half fibre  Fibreless particle, proximal half fibre $ \begin{array}{c ccccccccccccccccccccccccccccccccccc$

Defective lysate preparation, modification and complementations were carried out as described in the legend for Table 1, and the format is identical to that of Table 2. In addition to the mutants already described, (X4E: 34amB25/34am A455/35am B252/37am N52/38am B262) was used as a source of fibreless particles, free of fibre components.

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#### Interaction of dGMP radical with cysteamine and promethazine as possible model of DNA repair

THE lethal action of ionising radiation or alkylating drugs is thought to involve the formation of electrophilic species (many of which are free radical in nature) which interact with essential molecules such as nucleic acid. The protective effect of the nucleophilic compound, cysteamine, has been attributed to its ability, first, to scavenge these species thereby preventing their reaction or, second, to reduce chemically the free radical lesion thereby resulting in its repair1,2. A similar mechanism has been postulated to explain the protective effect of promethazine against carbon tetrachloride-induced liver damage3,4.

Although the scavenging actions of cysteamine (RSH) and promethazine (PZ) towards several electrophilic intermediates have been observed directly4-6, their reaction with nucleic acid related free radicals has not. Furthermore, it has been generally accepted that the initial products of reaction of the electrophilic hydroxyl radical with nucleic acid bases are adducts7.8. We here report experiments which indicate that in the case of guanine derivatives, radical-cations are formed in high yield and these can react stoichiometrically with the above nucleophiles. The pulse radiolysis technique using both optical4-6 and conductometric9 analysing facilities has been employed.

On pulse radiolysis of nitrous oxide saturated solutions of either thymidine, cytidine, adenosine or guanosine  $(6 \times 10^{-4} \text{ M})$ 

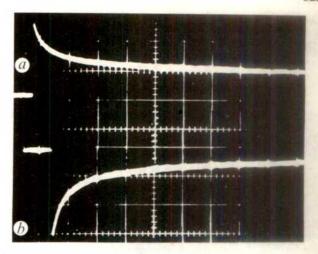


Fig. 1 Oscillograms showing changes in optical absorption and conductivity following pulse radiolysis of a nitrous oxide saturated solution of guanosine  $(6 \times 10^{-4} \text{ M}, pH = 4.6)$ . Dose about 2 k rad. a, absorption at 370 nm; b, conductivity. Time scale 1,000 µs per large division.

pH = 4-5) transient absorption spectra similar to those reported previously7,8 and attributable to the products of reactions of the hydroxyl radical, were observed. Except in the case of guanosine, little change in conductivity was observed over the same period. With the latter, a large decrease in conductivity occurred immediately after the radiation pulse. Such a change in conductivity in acid solution indicates the formation of positive ions and is attributed to the process:

$$G + OH \cdot \rightarrow G^{+} + OH^{-}$$
 (1)

where G = guanosine. The hydroxide ion is immediately neutralised:

$$OH^- + H^+ \rightarrow H_2O$$
 (2)

with the result that a highly conducting proton ( $\Lambda = 315 \Omega^{-1}$ cm<sup>2</sup>) is replaced by a less conducting cation ( $\Lambda = 50-70 \Omega^{-1}$ cm2) with a resultant decrease in conductivity. The subsequent increase in conductivity results from the liberation of a proton with the decay of the radical-cation. The optical signal at

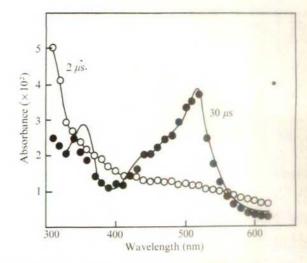


Fig. 2 Transient absorption changes observed on pulse radiolysis of nitrous oxide saturated solutions containing dGMP (10<sup>-2</sup> M) and promethazine (10<sup>-4</sup> M). ○, Experimental points taken after 2 μs; •, experimental points taken after 30 μs. The solid line through the 30 μs points is the absorption spectrum previously assigned to the promethazine hydroxyl radical adduct (see text).

370 nm measured simultaneously shows identical decay kinetics and is therefore attributed to the radical-cation (R.L.W., and K-D.A., unpublished).

In similar studies with slightly acid solutions of dGMP, radical cations were again detected. As in the case of guanosine the observable change in conductivity immediately after the radiation pulse, attributable to a reaction analogous to (1), decreased with increasing pH, no signal being observed at pH = 7. This does not, however, rule out the possibility of radical-cation formation in neutral solution. In the case of oxidised dGMP, in particular, the formation of a radical cation associated with a nitrogen atom and the simultaneous acid base dissociation of a phosphate residue would yield a zwitterion and would result in the absence of any accompanying change in conductivity. The fact that the transient optical absorption spectra of oxidised guanosine and dGMP were found to be similar in acid and neutral solution also provides support for the presence of similar species.

In the case of dGMP additional support for the formation of radical-cations is provided by studies with solutions containing excess bromide ions. Under the experimental conditions the electrophilic radicals Br2. were formed rapidly. These decayed rapidly and exponentially. The associated absorption was replaced by an absorption similar to that attributed to the radical-cation in accord with the reaction:

dGMP + Br<sub>2</sub>· 
$$\rightarrow$$
 dGMP· + + 2 Br - (3)  
 $k_3 = 4 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ 

On pulse radiolysis of nitrous oxide saturated solutions of 5' dGMP ( $10^{-2}$  M, pH = 7) and promethazine ( $25-100 \mu M$ ) the radical-cation absorption was again observed initially but decayed rapidly and was replaced by a new absorption,  $\lambda_{\text{max}} = 520 \text{ nm}$  (Fig. 2). A similar absorption at 520 nm was observed with solutions of guanosine. With thymidine, however, no such absorption was observed in agreement with the lack of formation of long-lived radical-cations on reaction of the base with OH. In the case of dGMP the rate of formation of the new absorption, which was of similar shape and magnitude to that of the radical resulting from direct interaction of the hydroxyl radical with promethazine, increased with increasing promethazine concentration according to first order kinetics.

The absolute rate constants for the reaction:

$$OH + dGMP \rightarrow dGMP + OH^{-}$$
 (4)

OH + PZ
$$\rightarrow$$
PZ (OH) · (5)  
 $k_4 = 5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1} \text{ and } k_5 = 1.0 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ 

have been measured (R.L.W., and K-D.A., unpublished and refs 10 and 11). As in the above experiment the concentration of dGMP > PZ the appearance of the 520 nm absorption must be due to reaction (4) followed by:

$$dGMP^{+} + PZ \rightarrow dGMP + PZ (OH) \cdot + H^{+} (6)$$
  
 $k_{6} = 2.8 \times 10^{9} M^{-1} s^{-1}$ 

Similarly on pulse radiolysis of solutions (pH 7.3) containing dGMP (10<sup>-2</sup> M) and cysteamine (0.2-1 mM) the exponential decay of the nucleotide radical absorption observed at 580 nm is attributed to the reaction:

$$dGMP^{+} + RSH (RS^{-}) \rightarrow dGMP + RS^{+} + H^{+}$$

$$k_{7} = 1.7 \times 10^{8} M^{-1} s^{-1}$$
(7)

These results show clearly that the direct interaction between nucleophilic agents and free radicals produced in nucleic acid can occur rapidly. As these radicals are intermediates in lesion production, the above processes constitute a 'repair' mechanism. Furthermore, although these observations have concerned the repair of lesions induced by the hydroxyl radical they could equally pertain to those induced by other electrophilic species such as those resulting from the biotransformation of carcinogenic or toxic agents, for example CCl3 ·, RNOH ·, or carbonium ions<sup>3,12</sup>.

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#### Human tumours in mice confirmed by chromosomal analysis

THE growth of human tumours in mice may be evaluated by node size and histological sections<sup>1-4</sup>. Because tumour cells may fuse with host cells5-7 in vivo, it is important to ascertain which cells produce the tumour. Human chromosomes may be identified using banding procedures and this makes it possible to verify that human cells alone grow in the tumour. If chromosomal analysis is performed on the tumour before injection, it would also be possible to determine which cells were able to grow rapidly in vivo. Here we present a report on such a chromosomal analysis of a human cervical carcinoma cell line grown in immunosuppressed mice and re-established in vitro.

Human cervical carcinoma cells were cultured from primary tissue by Sykes8. For this work they were cultivated in McCoy's medium with 20% foetal calf serum. The host animals were male 4-6 week old BALB/c/CRGL mice given 400, 500, or 600 rad of whole body X irradiation followed 2-3 h later with intraveneous bone marrow cells from syngeneic donors. The mice were inoculated subcutaneously in the flank with 106 human cells in a total volume of 0.05 ml of culture medium without serum 24 h after irradiation. When a nodule was felt or when 21 d after irradiation was approaching, the animal was killed and a culture was initiated with minced tumour. Three independent tumours from mice of different irradiation doses were studied. For chromosome analysis, G bands were done with trypsin and C bands were produced using alkaline 2X SSC (ref. 9).

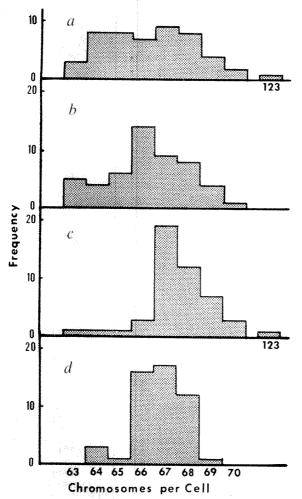
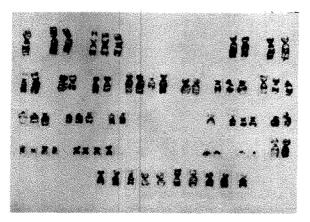


Fig. 1 Number of chromosomes counted in about 50 cells for each tumour, a, The original human cervical carcinoma cell line: b-d, cultures 1-3 (cell lines established from mousepassaged tumours taken from three separate animals).

Within 3 weeks nodules could be felt in most of the mice. These nodules measured 1  $\times$  2 mm to 4  $\times$  7 mm. Some animals were allowed to survive, with regression starting at 28-30 d after irradiation. When cultures were started at 12-21 d, the tumour cells grew well in culture.

At the time this cell line was first described, the chromosomal range of the cells was 48-130. At the time they were being grown for these experiments the cells had 63-70 chromosomes per cell with a mean of 66.1, a mode of 67 (18%), and 40% of these



Sykes tumour Giemsa-trypsin bands from a representative cell. Chromosomes were arranged according to normal and near-normal banding identification. The chromosomes on the last row showed too much rearrangement to allow positive classification.

cells having 66-68 chromosomes (Fig. 1a). After passage through mouse no. 1, culture 1 also had 63-70 chromosomes per cell with a mean of 65.6, a mode of 66 (28%) and 60% of the cells having 66-68 chromosomes (Fig. 1b). Culture 2 from mouse no. 2 had 63-70 chromosomes per cell with a mean of 67.5, a mode of 67 (38%) and 70% of the cells having 66–68 chromosomes (Fig. 1c). Culture 3 had a range of 64-69 chromosomes per cell with a mean of 66.7, a mode of 67 (34 $\frac{4}{9}$ ), and 90 $\frac{4}{9}$  of the cells having 66-68 chromosomes (Fig. 1d). The cells with 66-68 chromosomes seemingly had the capacity to grow without restraint. There were very few cells with less than 66 or more than 68 chromosomes after transplantation. Fifty cells were analysed from each tumour.

The original carcinoma line was examined by G and C bands to determine the chromosomal constitution. The majority of the chromosomes could be identified with normal or near-normal trypsin-induced banding patterns (Fig. 2). There seemed to be representatives of each human chromosome present in each cell. In cells with fewer chromosomes the A 1 was frequently absent with non-random losses from other groups. Several new arrangements were seen that could not be specifically identified as originating from particular chromosomes, but C band patterns verified that they were not mouse chromosomes. Several very small metacentrics were observed—seemingly from the fusion of 21 and 22 long arms. At least one X chromosome could be identified in most of the cells.

The three cultures examined after mouse passage did not show a marked difference in karyotypes. There did not seem to be any particular chromosomes lost or gained, although the variation from cell to cell in such a tumour makes this very difficult to ascertain. The chromosomes were all human, no mouse chromosomes were seen at all. In the three independent tumours the mean and mode of the parental line was born out as the prime growth cells.

Human tumour biopsies are frequently cultured to examine the chromosomes, do growth kinetics, and assay drug responses. There are frequently several cell types that grow from such biopsies. The animal passage of such a mixed culture can select and concentrate the tumour-producing cells as an in vivo cloning procedure. In this study, cells with 66–68 chromosomes had properties allowing unrestricted growth, and could therefore be still considered malignant. Most important, the tumour in the mouse was shown to be composed of the human cells that had been injected.

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#### Metabolic activation of benzo(a)pyrene proceeds by a diol-epoxide

CARCINOGENIC polycyclic hydrocarbons such as the widespread environmental contaminant, benzo(a)pyrene (Fig. 1a), undoubtedly require metabolic activitation. Boyland1 proposed that epoxides, whose formation is catalysed by the NADPH-dependent microsomal mono-oxygenases2,3, are the initial products of double-bond oxidation and we have suggested that epoxides are the important reactive metabolites responsible for the biological effects of these carcinogens4.

The properties of synthetic K-region epoxides lent some support to this hypothesis; they are alkylating agents that react covalently with nucleic acids, they are mutagenic in several systems and induce malignant transformation of rodent cells in culture<sup>5</sup>. Recent evidence suggests, however, that in cells treated with polycyclic hydrocarbons, it is not the Kregion epoxides that react with DNA6.

Here we report that in cells in culture or in model systems in vitro, benzo(a)pyrene seems to undergo a two-stage metabolic activation that initially involves the formation of the

Fig. 1 Benzo(a)pyrene (a) and 7,8-dihydro-7,8-dihydroxybenz (a) pyrene 9,10-oxide(b).

7,8-dihydrodiol<sup>7</sup>, which is then further metabolised on the isolated 9,10 double-bond by the microsomal mono-oxygenase to give 7,8-dihydro-7,8-dihydroxybenz(a)pyrene 9,10-oxide (Fig. 1b). Evidence that it is this type of diol-epoxide that actually reacts with DNA in cells treated with benzo(a)pyrene is also presented. As well as identifying, for the first time, the type of metabolite involved in the reactions of polycyclic hydrocarbons with genetic material, these findings also strongly support the general hypothesis that epoxides are ultimately responsible for the carcinogenicity of these compounds.

Primary cultures of Syrian hamster embryo cells were treated with benzo(a)pyrene labelled with either 3H or 14C. DNA was isolated from the cells, hydrolysed enzymically to nucleosides and the hydrolysates chromatographed on Sephadex LH20 columns eluted with methanol-water gradients6.8. It is already known that nucleosides are eluted in the early fractions, for example Fig. 2, peaks I, II, IV and V; Fig. 3a peaks I, II and IV and Fig. 3c peaks I and II and that polycyclic hydrocarbon-nucleoside products elute later as the concentration of methanol in the eluting solvent increases<sup>6,8</sup>. The elution profiles shown in Fig. 2 confirm that the DNAproducts formed in benzo(a)pyrene-treated cells are different from those formed in reactions of the K-region epoxide, benzo(a)pyrene 4,5-oxide, with DNA.

As there was some evidence that the further metabolism of dihydrodiol metabolites might be involved in the reactions of benzo(a)pyrene with DNA (ref. 9), 3H-labelled dihydrodiols were prepared in large-scale incubations of the radioactive hydrocarbon with rat-liver homogenate<sup>10</sup> and incubated in vitro with a rat-liver microsomal preparation and DNA11. The DNA was hydrolysed, mixed with a hydrolysate of DNA from cells treated with 14C-labelled benzo(a)pyrene and the mixtures chromatographed on Sephadex columns. Hydrolysates of DNA that had been incubated with microsomes and <sup>3</sup>H-labelled 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene gave elution profiles in which the major hydrocarbon-deoxyribonucleoside product eluted in a position similar to that occupied by the DNA product from 14C-labelled benzo(a)pyrene treated

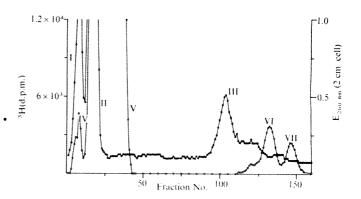
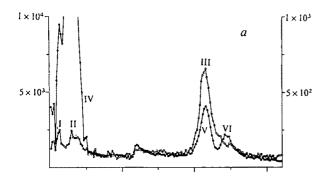
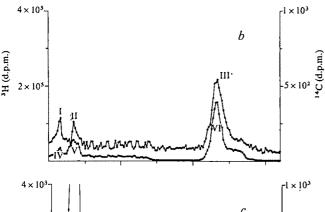


Fig. 2 Benzo(a)pyrene-deoxyribonucleoside products separated from hydrolysates of DNA by Sephadex LH20 column chromatography. •, Hamster embryo cell DNA from cells treated with <sup>3</sup>H-labelled benzo(a)pyrene; ○, DNA (salmon sperm) reacted with benzo(a)pyrene 4,5-oxide. The embryo cell DNA was obtained from primary cultures of Syrian hamster embryo cells grown in Dulbecco's MEM supplemented with foetal calf serum (15% v/v) in Thompson bottles (21) under 10% CO<sub>2</sub> in air. Confluent cell monolayers were treated for 24 h with  $^3$ H-labelled benzo(a)pyrene (1 µg ml<sup>-1</sup>) (specific activity 3.24 Ci mmol<sup>-1</sup>; the Radiochemical Centre, Amersham, UK) added as a solution in DMSO, final concentration 0.1%. Cells were collected with EDTA, the DNA isolated 15 and hydrolysed with DNase followed by phosphodiesterase and alkaline phosphatase<sup>6</sup>. Salmon sperm DNA (Type III, Sigma Chemical Co., St Louis, Mo.) (50 mg) in Tris buffer pH 7.4, 0.01 M (50 ml) was mixed with benzo(a)pyrene 4,5-oxide (2.5 mg) in ethanol (25 ml) and incubated at  $37^{\circ}$  C for 4 h. The reaction mixture was extracted with ether ( $3 \times 1$  volume), the DNA precipitated with ethanol (1 volume), washed in ethanol and ether and dried. Enzyme hydrolysates of this DNA and those of DNA from <sup>14</sup>C-benzo(a)pyrene treated cells were cochromatographed on a column (75 × 1.5 cm) of Sephadex LH20 that was eluted with a methanol-water gradient<sup>6,8</sup>. Fractions (5.3 ml) were collected and examined at 260 nm for ultraviolet absorbing materials; radioactivity was measured by liquid scintillation counting.

cells (Fig. 3a III and V). This finding was confirmed when DNA hydrolysates from embryo cells that had been treated with <sup>3</sup>H-labelled 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene were compared with hydrolysates from <sup>14</sup>C-labelled benzo(a)pyrenetreated cells (Fig. 3b, III and VI). Microsomal oxidation of the isolated 9,10-double bond present in the 7,8-diol of benzo(a) pyrene to give 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene 9,10oxide seemed likely to be the second metabolic step, especially since reactions with DNA did not occur if the dihydrodiol was incubated with microsomal preparations in the absence of the cofactors necessary for the NADPH-dependent mono-

Accordingly <sup>3</sup>H-labelled 7,8-dihydro-7,8-dihydroxybenzo(a) pyrene 9,10-oxide was synthesised and reacted with DNA and a hydrolysate compared on a Sephadex column with a hydrolysate of DNA from cells treated with 14C-labelled benzo(a) pyrene. The elution profile (Fig. 3c) obtained from DNA reacted with the diol-epoxide contained two peaks of radioactivity in the region normally occupied by hydrocarbondeoxyribonucleoside products, one of which (Fig. 3c, III) was





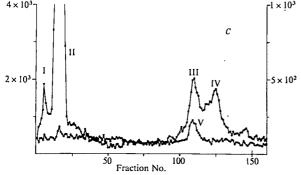


Fig. 3 Benzo(a)pyrene-deoxyribonucleoside products separated from hydrolysates of DNA by Sephadex LH20 column chromatography. a, O, DNA from hamster embryo cells treated with <sup>14</sup>C-benzo(a)pyrene (specific activity 21 mCi mmol<sup>-1</sup>). DNA was isolated and hydrolysed as described in the legend to Fig. 1.

•, DNA incubated with "H-7,8-dihydro-7,8-dihydroxybenzo" (a)pyrene in the presence of a rat liver microsomal preparation. DNA (salmon sperm) (60 mg) in Tris buffer pH 7.4, 0.01 M, (40 ml) was mixed with a washed rat liver microsomal preparation<sup>11</sup> (=5 g liver) resuspended in Tris buffer (40 ml) containing NADPH (24 mg), glucose 6-phosphate (120 mg) and glucose 6-phosphate dehydrogenase (14 units) to which <sup>3</sup>H-7,8-dihydro-7,8-dihydroxybenzo(a)pyrene (specific activity 437 mCi mmol<sup>-1</sup>) (43 µg) was added in acetone (0.2 ml). After incubation for 30 min at 37°, the microsomes were pelleted by centrifugation and the DNA reisolated and purified11. Hydrolysates of this DNA were cochromatographed with those from  $^{14}$ C-benzo(a) pyrene-treated cells. b,  $\bigcirc$ , DNA, from hamster embryo cells treated with  $^{11}$ C-benzo(a)pyrene, hydrolysed and cochromatographed with an hydrolysate of  $\bigcirc$ , DNA from hamster embryo cells treated similarly with  $^{3}$ H-7,8-dihydro-7,8-dihydroxybenzo (a)pyrene (specific activity 3.24 Ci mmol $^{-1}$ ) (0.25 µg per ml medium). c,  $\bigcirc$ , DNA from hamster embryo cells treated with  $^{14}$ C-benzo(a)pyrene hydrolysed and cochromatographed with an hydrolysate of  $\bigcirc$ , DNA reacted with  $^{3}$ H-7,8-dihydro-7,8-dihydroxybenzo(a)pyrene 9,10-oxide. The diol-epoxide was synthesised by treating the radioactive 7,8-dihydrodiol (235 µg) with m-chloroperoxybenzoic acid (200 µg) in chloroform (2 ml) at  $^{10}$ C for 48 h. After washing with NaOH and water, the chloroform solution was evaporated to dryness, the residue DNA were cochromatographed with those from <sup>14</sup>C-benzo(a) the chloroform solution was evaporated to dryness, the residue dissolved in ethanol and the epoxide purified by thin-layer chromatography on an Eastman-Kodak 6060 Chromogram developed with cyclohexane:dioxan, 1:1 (v/v). The diol-epoxide (specific activity 437 mCi mmol<sup>-1</sup>) (100 µg) in ethanol (1.5 ml) was mixed with DNA (salmon sperm) (3 mg) in Tris buffer (3 ml) and incubated, and the DNA reisolated and hydrolysed (see legend to Fig. 1).

coincident with the DNA product from <sup>14</sup>C-labelled benzo(a) pyrene-treated cells: the second product (Fig. 3c, IV) has not been further investigated. Small amounts of this second product seem to be formed when the 7,8-diol is incubated with microsomes and DNA (Fig. 3a, VI) but were not seen when either benzo(a)pyrene or the 7,8-diol were metabolised by embryo cells. The 14C- and the 3H-labelled hydrocarbon-deoxyribonucleoside products that eluted together from Sephadex columns (Fig. 3a, III and V; Fig. 3b, III and VI; Fig. 3c, III and V) were further examined on thin-layer chromatograms. Three dissimilar solvent systems failed to separate the 14C-labelled benzo(a)pyrene-deoxyribonucleoside products obtained from cells treated with the hydrocarbon from the 3H-labelled benzo(a)pyrene-deoxyribonucleoside products formed in the other experiments; in each case the 14C and the 3H-labelled products migrated together.

In other experiments, 3H-labelled samples of syntheticallyprepared 4,5- and metabolically-obtained 9,10-dihydrodiols of benzo(a)pyrene, which are also metabolites of this hydrocarbon7, were used. Hydrolysates of DNA from embryo cells treated with the 4,5- and 9,10-dihydrodiols did not appear to contain radioactive hydrocarbon-deoxyribonucleoside products although some were formed from the 9,10-derivative in microsomal incubations. Attempts to prepare 9,10-dihydro-9,10dihydroxybenzo(a)pyrene 7,8-oxide were not successful; the 9,10-double bond of benzo(a)pyrene seems to be chemically much less reactive than that in the 7,8-position.

Our results indicate that an isolated double-bond in the 9,10-position of benzo(a)pyrene leads to the metabolic formation of a 9,10-epoxide and that an epoxide formed in this position reacts effectively with cellular DNA. The dihydrodiols that are precursors of this type of epoxide are common as hydrocarbon metabolites and 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene has been detected as a benzo(a)pyrene metabolite that is formed by rodent liver and lung<sup>7,9</sup> and by human lymphocytes<sup>12</sup>. Preliminary experiments with other hydrocarbons imply that analogous metabolic activation mechanisms also apply, for example, to benz(a)anthracene and to 7-methylbenz(a)anthracene. They also indicate that diol-epoxides of the type discussed here may be effective in reaching cellular DNA simply because diol-epoxides are not good substrates either for the epoxide hydrase<sup>13</sup> or for the glutathione transferase<sup>11</sup>, enzymes that normally inactivate epoxides. The work carried out so far indicates that diol-epoxides like 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene 9,10-oxide are important in the metabolic activation of the polycyclic hydrocarbons to intermediates that react with DNA; consequently they might be expected to be biologically active and appropriate tests for carcinogenicity and mutagenicity are in progress.

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#### Babesia microti and Plasmodium berghei voelii infections in nude mice

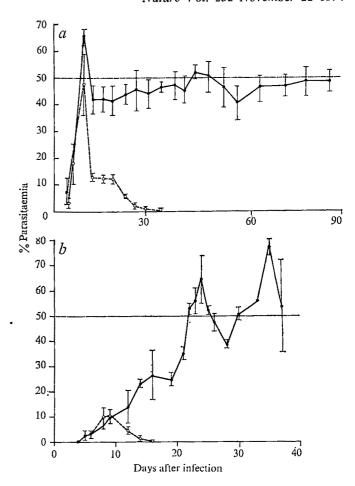
THE precise nature of the responses to Babesia spp. and Plasmodium spp. which lead to their elimination from the host and subsequent acquisition of immunity is uncertain. A degree of thymus dependence is involved, as shown by the aggravation of infection by neonatal thymectomy1,2, anti-thymocyte serum3 and anti-lymphocyte serum4.5. For instance, parasitaemias due to P. berghei were higher in neonatally thymectomised rats than in controls, and lasted about twice as long, with higher mortality1,2.

The recent availability of congenitally hypothymic (nude nu/nu) mice allows a more critical appraisal of the importance of the role of thymus competence in the elimination of these parasites. B. microti and P. berghei yoelii produce transient parasitaemias in normal mice, followed by recovery and resistance to challenge with the same species of parasite<sup>6,7</sup>. We have investigated these infections in nude mice.

Groups of three nude mice or normal littermates 6-8 weeks of age were infected by intraperitoneal inoculation with 108 erythrocytes parasitised by either B. microti (King's 67 strain, provided by Dr F. E. G. Cox) or P. berghei yoelii (17 × strain, from Dr N. Wedderburn). The nude mice were housed in germ-free isolators. Tail blood smears were stained with Giemsa, and the number of parasitised cells in 400 erythrocytes counted. The course of the B. microti infection is summarised in Fig. 1a, and of P. berghei yoelii in Fig. 1b. Means and ranges are indicated.

Following a latent period that was the same in all cases, there was a transient parasitaemia in littermate control mice and a persistent parasitaemia in nude mice. Apparent differences in the nature of this persistence of each parasite can be explained in terms of preference for different developmental stages of the red cell series, for whereas B. microti seems largely to be restricted to mature red cells P. berghei yoelii parasitises only reticulocytes. Nearly all the reticulocytes in the P. berghei yoelii infected nude mice contained parasites from day 10. Thus the rise in reticulocyte number with worsening anaemia, rather than any difference in the response of nude mice to the two organisms, is likely to be responsible for the overall parasitaemia remaining stable with one (Fig. 1a) and increasing with the other (Fig. 1b). A high and stable percentage of the susceptible red cell stage was infected by each organism until the nude mice eventually died or were killed.

In contrast to our findings, neonatally thymectomised rats took only twice as long as normal littermates to clear P. berghei from the peripheral circulation<sup>1,2</sup>. There is evidence that neonatal thymectomy in rats does not completely eliminate T lymphocytes8. Thus, in keeping with data obtained with anti-thymocyte serum3, the thymus dependence of elimination of Plasmodium spp. from rodents appears to be more complete than earlier work suggests.



a, Babesia microti infection in nude mice and normal littermates. b, Plasmodium berghei yoelii infection in nude mice and normal littermates. •, nu/nu; O, nu/+.

The results obtained with B. microti in nude mice (Fig. 1a) were similar to those found on giving anti-lymphocyte serum to hamsters before and during infection with this parasite<sup>5</sup>. This confirms an absolute dependence on the thymus for elimination of this parasite as well as P. berghei yoelii. Nude mice may be a more suitable model because they seem to survive persistent parasitaemias of B. microti more successfully than do antilymphocyte serum treated hamsters. On day 80, twice as long as any hamster survived, nude mice were apparently quite healthy after maintaining a 50% parasitaemia for the previous 70 d. In addition the problem of anti-lymphocyte serum standardisation inherent in the hamster model is eliminated.

In summary, the present data confirm that elimination of B. microti and P. berghei yoelii from the circulation of mice does not occur without thymus competence. It remains to be established whether the thymus-derived cells exert a helper effect in the production of a particular class or subclass of antibody required for parasite elimination, or form part of another mechanism of immunity. Further experiments are in progress to test these possibilities. Clearly the parasitised nude mouse could prove to be a useful model for further immunological research in this field.

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#### Evidence for oligomeric IgA production by peripheral rat lymph nodes

A DISTINCTIVE characteristic of IgA is its polymorphic nature with regard to the molecular size. It occurs in secretions mostly as a dimer or tetramer<sup>1,2</sup> while serum IgA has a polydisperse weight distribution in many species<sup>1</sup>. It is mostly monomeric in human serum (7S) (ref. 3), mostly polymeric in the mouse<sup>4</sup> and in the dog<sup>5</sup>, but in both these and other species several molecular sizes occur in the blood<sup>1</sup>.

When extending studies on natural anti-hapten antibodies in the rat<sup>6</sup> we made the unexpected finding that natural anti-3-iodo-4-hydroxy-5-nitrophenylacetyl (NIP) of the thoracic duct lymph had sedimentation characteristics (9S–13S) similar to the secretory IgA antibodies that we could demonstrate in rabbit colostrum<sup>7</sup> and to the oligomeric IgA produced by mouse spleen cultures<sup>8</sup>. Natural antibody in the blood serum of the same rats sedimented like 19S IgM. This finding prompted us to study sedimentation patterns of anti-NIP antibodies in the blood and the lymph of immunised rats.

Rats were immunised with 10 µg NIP-SIII pneumococcal polysaccharide<sup>8</sup> or 500 µg of NIP chicken globulin (CG)<sup>8</sup> in complete Freund's adjuvant (CFA). As we wanted the antibody production to take place preferentially in the peripheral lymph nodes draining to the thoracic duct the antigen was injected into the hind foot pads. The rats were bled and cannulated (abdominal thoracic duct) 8–9 d after immunisation. Thoracic duct lymph samples were clarified by spinning for one hour at 50,000g. The lymph and the serum samples were titrated with NIP-T<sub>4</sub> bacteriophages as described earlier<sup>9</sup>. They were centrifuged through a continuous and linear 5–20% sucrose gradient in 12 ml tubes in rotor SW-41 Ti of a Beckman L 3–50 ultracentrifuge for 14 h (39,000 r.p.m.). A total of 25 fractions were collected and titrated with NIP-T<sub>4</sub>. Sedimentation constants (S<sub>20</sub>, w) were derived according to McEwen<sup>10</sup>.

The predominant antibody peaks of the blood serum had sedimentation constants of 19S and 7S. A minor 9S component could usually be detected (Fig. 1a). Antibodies of the thoracic duct had major 19S and 7S fractions but they also had considerable 13S and 11S fractions (Fig. 1b); 9S antibody could be seen as a distinct peak in some but not all lymph samples. We have found this phenomenon in all the 14 serum-lymph pairs studied so far.

In some experiments we incubated diluted lymph before centrifugation with rabbit anti-rat IgA. Anti-IgA is known to

Table 1 Characteristics of the antibody classes that could be separated from the thoracic duct of immunised rats and lymph node culture fluids

Estimated			Molecular weight of the theoretical human IgA polymer that would come closest
Stokes'		Determined	to the observed
radius	Ig	molecular	molecular weight
(Å)	class	weight	(see text)
107	IgM	860,000	` ,
91	ΙgΑ	515,000	520,000
80		380,000	370,000
70	IgA	280,000	320,000
	(Å) 107 91 80	Stokes' radius Ig (Å) class 107 IgM 91 IgA 80 IgA	Stokes'         Determined molecular molecular           (Å)         class weight           107         IgM         860,000           91         IgA         515,000           80         IgA         380,000

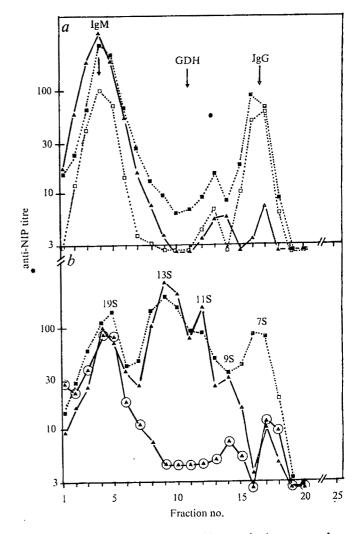


Fig. 1 Sedimentation pattern of anti-NIP, a, in the serum and, b, thoracic duct lymph of rats after immunisation with NIP-CG and NIP-SIII.

NIP-CG immunisation; ..., NIP-SHI/immunisation; encircled triangles, sample mixed with anti-IgA. Different symbols indicate different rats. Rabbit anti-DNP and glutamate dehydrogenase (GDH) were used as reference compounds, their maximum activity is indicated by arrows.

displace or eliminate IgA but not IgG or IgM antibodies in the sedimentation pattern<sup>8</sup>. Data in Fig. 1 show that the 9S, 11S and 13S peaks were displaced by our anti-IgA and were thus very probably IgA.

The total IgA of several lymphs and sera was determined by the method of Mancini, Carbonara and Heremans<sup>11</sup>. We found that concentrations in the lymph fluid were 4–12 times higher than in the serum of the same animal. In the ultracentrifugation the IgA of the lymph fluids was fairly evenly distributed from the 7S to 15S fractions while the serum IgA was distributed from the 7S to 11S fractions.

The Stokes's radii of the four heaviest antibody fractions were determined by gel filtration according to Laurent and Killander<sup>12</sup>. They were calculated as means of two gel filtration runs, through a Sepharose 6B column  $(1.5 \times 79 \text{ cm})$ . Reference proteins in these runs included rat serum albumin, hyperimmune rabbit anti-DNP (IgG) and glutamate dehydrogenase. Their radii were assumed to be 35,51 and 60 Å respectively<sup>13,14</sup>. The fractions from the gel filtration were rerun in ultracentrifugation. Molecular weights (Table 1) were calculated using the  $S_{20, w}$  values and the Stokes radii in the formula

$$M = (6\pi\eta_{20, w} N a_{20, w})/(1 - \bar{\nu}\rho_{20, w})$$

in which  $\eta_{20, w}$  and  $\rho_{20, w}$  are the viscosity and density of water at 20° C, N is Avogardo's constant and a is Stokes's radius. The

partial specific volume (v) of oligomeric IgA was assumed to be 0.73 (ref. 15).

It is possible that 9S-13S IgA anti-NIP of the thoracic duct lymph mainly originates in the intestinal lymphoid tissue. For instance, plasmablasts generated in the regional lymph nodes may be transferred to the intestinal area and produce antibody there<sup>16</sup>. It was made unlikely, however, by the finding that when popliteal lymph nodes were removed from these rats and kept in organ cultures for 6 h without additional antigen they produced the same 9S-13S fractions which we found in the thoracic duct lymph. These again could be displaced by anti-IgA (Fig. 2).

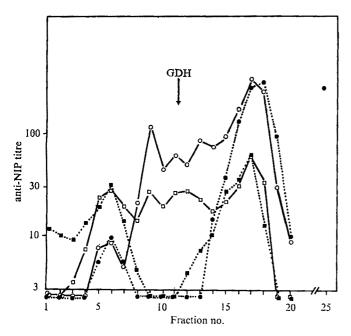


Fig. 2 Sedimentation pattern of anti-NIP activity produced by were cultured for 6 h (□, ■) or 24 h (○, ●) without additional antigen. □ and ○, untreated samples; ■ and ●, samples mixed with anti-IgA, the rat represented by open squares is represented in the same way in Fig. 1. in the same way in Fig. 1.

Data in Fig. 2 are derived from the first two lymph node cultures we tested but we have studied five nodes from five rats. Four nodes produced the 9S-13S IgA antibody. One node produced little antibody, and all of it was 19S. These data suggest that at least some 9S-13S IgA anti-NIP was produced by regional lymph nodes of our rats.

We considered the possibility that the polymerisation had taken place outside the synthesising organs. The following facts, however, fail to support it. First, individual lymph samples maintained their sedimentation pattern through weeks of storage at  $-20^{\circ}$  C. Second, centrifugation of lymph 15 min after it had left the thoracic duct showed a similar sedimentation pattern to lymph samples centrifuged later (rat no. 5 marked with closed squares in Fig. 1b). Third, in vitro synthesis of 9S-13S IgA antibody by lymph node fragments.

We believe that the different IgA antibody fractions represent different degrees of polymerisation. The molecular formulae of the three IgA fractions cannot be derived from our data but if we make the assumption that the molecular weight of the light chain, the a chain, J piece, secretory piece, and the carbohydrate content are the same in the rat IgA as those in man we find that our observed molecular weights would correspond reasonably well with the theoretical molecular weights of the following reconstructed human polymers; trimer+secretory piece+J piece (our 13S IgA), a dimer+secretory piece+J piece (our 11S IgA) and dimer+J piece (our 9S IgA).

We conclude that non-intestinal lymphoid tissues produce

polymerised IgA antibodies in immunised rats. This antibody can be demonstrated in the thoracic duct lymph and cultures of popliteal lymph nodes. It occurs in three main size classes with molecular weights of approximately 515,000, 380,000 and 280,000. In the blood these antibodies, especially the two heavier classes, are either rapidly catabolised, reduced to small subunits, or secreted.

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#### Antigenic inhibition of cell-mediated cytotoxicity against tumour cells

THERE is now abundant evidence that tumour cells express new surface antigens (tumour-associated antigens, TAA)1. In many tumour models, cell-mediated immunity directed against TAA has been demonstrated in vitro2. Serum from tumour-bearing individuals has been shown to inhibit specifically cell-mediated immune responses (CMI) to the tumour target cells in vitro3, and this phenomenon may explain the paradoxical coexistence of a growing tumour and an immune response directed against the tumour. Both immune complexes of TAA with antibody4,5, and soluble TAA alone<sup>6</sup>, have been implicated as inhibitors in these sera.

In most studies, however, neither the nature of the effector cell nor the mechanism of inhibition, have been well characterised. CMI against tumours induced in mice by murine sarcoma virus (MuSV), which was measured in a microcytotoxicity assay (MCA), and in which both T and non-T effector cells were cytotoxic, was inhibited by progressor serum and by soluble TAA7, In concurrent studies with a short term 51Cr release assay, in which only T cells were cytotoxic, there was no inhibition. In antiallogeneic CMI, cytotoxicity by T cells

Table 1 Effect of tu	mour-b	earer sera	on CMI
Origin of Serum		% Inhibit	ion of specific lysis*
Hyperimmune animals		-8	(15.2/14.1)
Regressor animals		5	(22.0/23.0)
_		4	(13.5/14.1)
		14	(12.3/14.1)
		17	(11.7/14.1)
Progressor animals			
Intact		30	(9.9/14.1)
		36	(9.1/14.1)
		47	(7.5/14.1)
		48	(7.3/14.1)
		83	(2.3/14.1)
Immunosuppressed	800 r. 800 r.	52 35	(11.0/23.0) (15.0/23.0)
Anti-lymphocyte globulin	000 I.	36	(14.7/23.0)

5×10<sup>6</sup> spleen cells from 10 d immune rats in cold RPMI-1640 5×10<sup>6</sup> spleen cells from 10 d immune rats in cold RPMI-1640 supplemented with HEPES buffer (20 mM) and 10% heat inactivated foetal calf serum, were added to 5×10<sup>4</sup> <sup>51</sup>Cr-labelled W/FuG-1 lymphoma cells (an *in vitro* adapted line), in the 16 mm wells of Linbro trays. After 4.5 h at 37° C with rocking (six to and fro tilts through 15° per min), <sup>51</sup>Cr release was determined and the specific release calculated <sup>10</sup>. Controls included spleen cells from age-matched normal rats and target cells incubated alone. Maximum releasable courts were those released following the incubation of releasable counts were those released following the incubation of target cells in 1.5% Brij detergent.

% Specific release of  ${}^{51}$ Cr =  $\frac{1}{(\text{Detergent c.p.m.} - N c.p.m.)}$ I c.p.m. - N c.p.m. $\times 100\%$ where I and N are the counts released by immune and normal cells. Spontaneous release was usually 15-20% and the release by normal cells, 25-35% of the detergent release respectively. % inhibition =  $I-I_{l,nblb} \times 100$ , where I and  $I_{l,nblb}$  are the % specific release by

where I and  $I_{Inhib}$  are the % specific release by immune cells in the absence or presence of inhibitor respectively. Sera, including normal rat serum used as a control, were heat inactivated and added at a final concentration of 1:5 to lymphocytes and target cells, and to target cells alone. Irradiation was administered from a 60Co source. Anti lymphocyte globulin was made in pigs to rat thymocytes. Rats received 50 mg subcutaneously on days -4 and 0.
\*Immune cells+inhibitor/immune cells only.

could<sup>8</sup> or could not<sup>9</sup> be inhibited by soluble fractions containing H-2 antigen.

The syngeneic transplantable Gross virus-induced lymphomas (C58NT)D and W/FuG-1 are rejected after subcutaneous injection within 10-15 d in about 85% of adult W/Fu rats10. Progressive, lethal tumour growth is observed following intraperitoneal injection of (C58NT)D cells, when the tumour grows in ascitic form, or following the subcutaneous injection of either tumour in immunosuppressed rats. The CMI response of rats to these tumours, as measured in a <sup>51</sup>Cr-release assay, is mediated by T cells<sup>10-12</sup>. Here we report the inhibition of cytotoxicity by progressor sera and also by cell-free TAA.

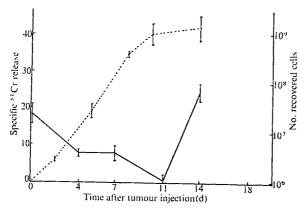


Fig. 1 Effect of progressor serum on CMI. Sera taken at various times from a group of six rats injected with 10<sup>6</sup> (C58NT)D cells intraperitoneally, were tested at a dilution of 1:5, for inhibition of CMI with 10 d immune spleen cells and <sup>51</sup>Cr-labelled W/FuG-1 cells (cell ratio 100:1). All other experimental conditions were as described in Table 1.—, % specific stor release; ---, No. recovered cells. Standard errors are indicated.

All inhibitors were tested in a 4.5 h 51Cr release assay using immune spleen cells taken 10 d following the subcutaneously injection of 108 (C58NT)D cells into adult inbred W/Fu rats10,13.

In these experiments progressive growth of tumours was obtained following the subcutaneous injection of 108 W/FuG-1 cells into immunosuppressed rats, and occasionally in normal rats. Tumour diameters were measured regularly to define the progressive and regressive phases of growth.

In Table 1, hyperimmune serum (raised by multiple injection of 107 W/FuG-1 lymphoma cells) is compared with progressor and regressor sera from the five adult rats, out of an injected group of 31, which showed tumour growth beyond 20 d. Progressor sera were taken during the phase of progressive growth between 20 and 30 d and regressor sera were taken from the same rats during regression of the tumours. Progressor sera from immunosuppressed rats were taken at 14-20 d or just before death.

Table 2 Inhibition of CMI by TAA

Table 2 II	minormon or c	MII DY	IAA			
Antigen	%	Inhibit dose	ion o			is
	400	100	50	30	10	3
(C58NT)D extract* MuLV-G	73	42 54	39 88	39 31	22 9	3
MuLV-G F/T† MuLV-M MuSV-M		44	00	31	14	
MuLV-G formal‡ p31		31 47		19	11	9

Immune spleen cells (5×106) were incubated with antigens for the duration of the assay. The antigens were added in 0.1 ml volumes and were also tested on target cells only. As a control, an equivalent volume of PBS was added to both lymphocytes and target cells, and to target cells only. The Gross and Moloney pseudo-types of murine leukaemia virus, MuLV (MuLV-G and MuLV-M) were purified by differential centrifugation from in vitro culture supernatants of W/Fu Gross and Moloney virus-induced lymphomas. MuSV-M was obtained from cultures of a BALB/c mouse sarcoma line (MSC)<sup>17</sup>. p31 was prepared from MuSV-M by an isoelectric focusing method<sup>18</sup>. Crude soluble extracts were made of (C58NT)D cells and normal rat spleen cells by papain digestion<sup>19</sup>. All reagents were dialysed exhaustively against phosphate buffered saline (PBS) before The results are the means of several experiments. All reagents in Tables 2 and 3 were tested at least twice and mostly three to four times. In no case was the standard error of the mean % inhibition greater than 5%. The mean of the % specific cytotoxicity, without added inhibitors, in these experiments was  $28\pm3\%$  s.e. Antigen concentrations were calculated by the method of Lowry<sup>23</sup> using bovine serum albumin as standard.

\*Crude soluble fraction of a papain digest.

†Eight freeze-thaw cycles. ‡Formalised virus, 0.01% formalin, 4° C for 2 weeks (ref. 20).

Statistically significant ( $P \le 0.01$ ) inhibition of CMI is seen with all progressor sera by comparison with regressor sera. Normal rat serum itself exerts an apparent small inhibitory effect, largely due to the lower spontaneous release of 51Cr in the presence of rat serum. When sera were tested at dilutions up to 1:10,000, augmentation of CMI was observed with hyperimmune and regressor sera, but not with progressor sera. The augmentation may reflect cytotoxicity by non-immune cells in the presence of an antibody in hyperimmune and regressor sera (lymphocyte dependent antibody)14 which, characteristically, is effective at high dilution<sup>15</sup>. The inhibitory effect of progressor sera was lost at dilutions greater than 1:20. Where sequential bleeds were taken during progression or regression of the tumour, the % inhibition of CMI was directly related to tumour diameter.

In Fig. 1, sera from adult rats with a progressive ascites lymphoma (106 (C58NT)D cells injected intraperitoneally) were tested for inhibition of cytoxicity at various times during tumour growth. The rats died at a mean of 14.7 d. Tumour growth was measured by the number of cells recovered from the peritoneal cavity. Increasing inhibition of CMI by serum

on days 4, 7 and 11 ( $P \le 0.01$ ) is seen during the tumour growth, but inhibition is lost just before death when tumour growth rate has decreased. The characterisation of the inhibitor in serum is in progress.

To test the effect of cell-free antigen alone on CMI, whole virus, the major viral group specific antigen p31 (ref. 16), and a soluble papain digest of tumour membrane, were used.

The results (Table 2) show that equal amounts of both the Gross and Moloney pseudotypes of MuLV, and formalintreated non-infectious MuLV-G, produce approximately the same inhibition of CMI. The inhibition by formalin-inactivated virus suggests that inhibition does not result from viral infection of effector lymphocytes. The group specific inhibition by virus may reflect a degree of particle disruption during virus purification with release of the internal group-specific antigens. The most abundant of these, p31, has been detected in virus stocks (R.A.K., unpublished). Furthermore, an aliquot of MuLV-G intentionally disrupted by eight freeze-thaw cycles is 2.3 times as potent as the same undisrupted virus stock (89% against 39% inhibition—Table 2). Purified p31 itself inhibits cytotoxicity.

Tabl	e 3	Specificity of inhibition		
a CMI to (C58NT)D* Antigen	ı	% Inhibition of specific dose of antigen (μ 1000 400 100 30 10	g)	is 1
Normal spleen extract Bovine serum albumir Newcastle Disease Vir MuLV-G	1	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	3	1 2
b CMI to L5178Y† Ratio 200:1 100:1 50:1	Ad	ded antigen (µg) % Speci 49. 16.	5 0	⁄sis
100:1 100:1 100:1 100:1 100:1	Mu Mu (C5	LV-G 100 15. SV-M 200 16. SV-M 100 16. 8NT)D extract 300 15. 8NT)D extract 150 14.	7 3 7 9	

\*CMI performed with 5×106 lymphocytes (that is, ratio of 100:1). †Normal or immune spleen cells from W/Fu rats immunised 7 d previously with  $2\times10^8$  murine L5178Y lymphoma cells intraperitoneally, were assayed with  $5\times10^4$  <sup>51</sup>Cr-labelled L5178Y cells for 8 h at 37° C with rocking. The results of a single typical experiment are

T lymphocytes, purified from L5178Y immune rat spleen cells by nylon column fractionation, are cytotoxic for <sup>51</sup>Cr-labelled L5178Y lymphoma cells. At a ratio of 100:1 the specific cytotoxicity of fractionated cells was 12.7%, and that of the unfractionated cells was 7.1%, in a typical experiment.

The inhibition by virus and viral antigens is specific (Table 3a). It can be seen that an antigenically unrelated virus, Newcastle Disease Virus, and soluble antigens from normal rat spleen cells do not inhibit CMI in comparison with MuLV-G. A syngeneic CMI response in the rat directed against TAA induced by an unrelated virus and measured in a short term 51Cr release assay, would have been the ideal control for the specificity of inhibition of CMI. In the absence of such a system, MuLV-G, MuSV-M and (C58NT)D extract were tested in an anti-xenogeneic system (Table 3b). The spleen cells of rats immunised intraperitoneally with  $2 \times 10^8$  murine L5178Y lymphoma cells 7 d previously, exhibit marked CMI towards 51Cr-labelled L5178Y cells in an 8 h assay, but this response was not inhibited by the added TAA.

Preliminary experiments suggest that the inhibition of CMI by cell-free TAA is at the level of the effector cell. The cytotoxicity of spleen cells was inhibited by 30% following preincubation with MuSV-M before assay (100 µg per  $5 \times 10^6$  cells). Preincubation of target cells with antigen did not alter the cytotoxicity.

These experiments were performed with unfractionated

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immune spleen cells, in which T cells have been shown to be the effector cells<sup>10-12</sup>. To substantiate the direct inhibition of T effector cells by antigen, B cells and macrophages were removed by incubation of immune spleen cells on nylon wool columns<sup>21</sup>. Phagocytic cells are regularly reduced from 7-9% of total cells to about 0.5% by this procedure10. The specific cytotoxicity by column purified cells at a ratio of 100:1 (in which the percentage of immunoglobulin bearing cells had been reduced from 40% to 4%) was 35%, and was inhibited by MuSV-M virus disrupted by freeze-thawing (40 µg produced 35% specific inhibition), and by a papain digest of (C58NT)D lymphoma cells (350 µg inhibited 96%). The same spleen cell population, tested without fractionation, was also inhibited by the same dose of these antigens.

Our evidence indicates that effector T cells can be inhibited in a 51Cr release assay by progressor sera and by cell-free TAA. This differs from results obtained in the MuSV tumour model in mice where CMI was inhibited by TAA and progressor serum only in MCA, in which both T and non-T cells were cytotoxic7, and not in a 51Cr release assay. This may reflect species differences in functional accessibility of receptors to the antigens studied. Our results indicate that the expression of a cell-mediated immune response can be inhibited by antigen at the level of the final effector cell. A recent report<sup>22</sup> indicates that antigen can also inhibit the release of antibody from antibody forming cells.

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\*First described by A.L.STEINER et al., Biochemica et Biophysica Acta 276, 155-161 (1972) Proc. Nat. Acad. Sci. 64, 367 (1969). Cholesterol oxidase Cat.No. 15626 Phospholipase C Cat.No 15636 from Nocardia erythropolis E.C. 1.1.3.6. trom Nocardia erythropolis E.C. 1.1.3 Specific activity: approx. 2DU/mg Only 0.1U of enzyme is needed for assay of approx. 5mMoles/litre cholesterol. Of particular interest to food analysts, and researchers and research clinical. When elucidating the type and arrangement of membrane phospholipid structures in erythrocytes the enzyme from B, cereus has the protesses, hydrolases neuraminidase and has negligible hasmolytic activity. rence: J.SHILOACH et al., Biotechnol and Bigengen XV, 551 (1973)

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# reviews

The Interrelationships of Fishes is a very welcome volume, for it contains an up to date statement of position by leading authorities on nearly every major group of fishes and, therefore, it provides an almost comprehensive survey of the state of palaeoichthyology in 1972. Tipping the scales at three and a half pounds it is in every sense a heavyweight volume, but its publishers and editors are to be congratulated on an attractive format and a text that is almost free of blemishes.

The interrelationships of most of the groups of fish have long been uncertain. That is partly because most major groups appear suddenly, already fully distinct, in the Devonian, and partly because of the rapid and complex evolution that characterised the emergence and early evolution of the teleosts. Though some new discoveries, and even more new opinions, have appeared in the last few years, it has been difficult for the non-ichthyologist to gauge the extent to which these have been accepted, especially in their implications for the major dichotomies in the evolution of fishes.

'Dichotomies' is the obligatory term, for the procedures of phyletic analysis advocated by Hennig have penetrated more thoroughly into the world of palaeoichthyologists than into other fields of vertebrate palaeontology, and the book is notable for its uniformity of approach in such matters. Irrespective of the merits or demerits of these methods, the uniformity does make it easier to analyse the precise points at issue where opinions differ.

This well produced and carefully edited book accurately reflects current American practice in the development of hormone radioimmunoassays. Each hormone, or group of hormones, is dealt Methods of Hormone Radioimmunowith separately, with particular emphasis being given to the particular Behrman. Pp. xxi+520. (Academic: methodological problems likely to be New York and London, May 1974.) encountered. Thus the book makes an ideal companion to previous publications concerned with the practicalities field and individual chapters deal with Some chapters, such as that relating of radioimmunoassay, which consider hapten radioimmunoassays for the in- to the radioimmunoassay of ACTH, the subject under such general sections tracellular messengers (cyclic AMP as, for example, radioiodation, separa- and cyclic GMP), for three of the mation which would be easy to reprotion and antibody production. It is prostaglandins, for the adrenal and unfortunate, therefore, that the exces- gonadal steroids and for the thyroid sive price of the book will limit its pur- hormones. Other chapters relate to book is that the authors have summarchase, other than by large specialised the assay of human placental lactogen, ised the assay kits and reagents availcentres.

more, authorities in the appropriate hypothalmic releasing hormones (TRH obtained.

# Fish families

#### **Barry Cox**

Interrelationships of Fishes. Edited by P. H. Greenwood, R. S. Miles, and Colin Patterson. (Supplement No. 1 to the Zoological Journal of the Linnean Society.) Pp. xiv+536. (Academic: London and New York, January 1974; Published for the Linnean Society.) £11.00; \$31.00.

Some of the papers in this volume contain radically new views resulting from new information. For example, the increased knowledge of the onychodont crossopterygians leads Andrews to question whether the coelacanth is isolated from all other crossopterygians. She suggests instead that the primary division of the group is an osteolepid-rhizodont-onychodont assemblage and a coelacanthporolepid assemblage. By way of contrast, Bjerring considers that the coelacanths do not share any signifificant specialisations with any other group of fishes and that their origin is remote from that of the other groups.

Other new discoveries from the Carboniferous of North America, described by Zangerl, have transformed theories of the phylogeny of the chondrichthyes. There was clearly already a considerable diversity of elasmobranchs in the Carboniferous

the bizarre iniopterygians Period: show both chimaeroid and elasmobranch characteristics, and the simple view that the two groups are members of a monophyletic chondrichthyan group is again in the ascendant.

New information on the early, Upper Devonian, actinopterygians is used to substantiate current views on the interrelationships of the Actinopterygii, Crossopterygii, Dipnoi and Acanthodii. The relationships of the Acanthodii, as evidenced by the exoskeleton of the head, is also discussed in a succinct and cogent paper which concludes that they are more closely related to osteichthyans than to chondrichthyans.

Many papers involve the use of from morphological information already known groups, using Hennig's methods, to ascertain the most likely scheme of relationships. In varying degrees of details, these methods are used for the chondrosteans, the holosteans (which are found to be a paraphyletic group, and discarded), the osteoglossomorph teleosts, the clupeomorphs, the elopomorphs, the ostariophysans and the higher euteleosts. Finally, there is a new classification of living elasmobranchs, including all the different genera, and the interrelationships of the four defined superorders are dis-

The addition of a citation index and a subject index add greatly to the utility of the volume, which is a worthy tribute to Professors Stensïo and Jarvik, in whose honour the 1972 symposium was held.

# **Detecting hormones**

J. Landon

assay. Edited by B. M. Jaffe and H. R. \$29.00; £13.90.

human chorionic gonadotrophin, the able for each hormone, and listed the Each chapter is written by one, or known pituitary hormones, two of the address from where they can be

and LH-RH), the gastrointestinal peptides, parathyroid hormone and the vasoactive peptides, including bradykinin, renin and the two angiotensins.

The quality of the individual chapters varies considerably, as is inevitable with a multiauthor book. The general standard is, however, high; the book is an excellent source of relevant references and the editors have ensured continuity. contain a wealth of practical inforduce.

A novel and useful feature of the

# No enemy but winter and rough weather

#### Television Review by Allan Piper

On Wednesday evening, immediately following The Frost Interview, the BBC broadcast its much heralded, prestige extravaganza The Weather Machine (BBC2, November 20, 9.00 p.m.); the latest in a series of annual productions which began so successfully back in 1970 with Violent Universe. Excellently assisted by the studio commentary of Magnus Magnusson, the modulated narrative tones of Eric Porter and, more importantly, by the availability of a six figure budget, producer Alec Nisbett endeavoured to squeeze into 120 minutes of airspace the fruits of twelve months globetrotting.

The programme followed much the same format as its predecessors, with periods of studio commentary (which saw Magnusson perched uncomfortably atop a large model world looking as though he might indulge in some involuntary globetrotting himself) followed by long snippets of film. All in all, very similar to the university lectures I remember. The similarity was probably intentional and, considering the objectives of the programme, was a good idea.

The Weather Machine, like its predecessors, was scripted by Nigel Calder. But even though his undeniable competence always shone through (he was winner of the UNESCO prize for the popularisation of science in 1972) my sense of enlightenment was somewhat tempered by the banjaxed mood in which I found myself once it was all over. The production set itself too many goals; it is simply not possible for anyone comprehensively to cover so much ground in so short a time. We saw ice sheets in Greenland and tornados in America and buoys in the Pacific and some floods in Japan and a mountain in Hawaii and Magnus Magnusson orbiting Venus.

And out of it all we learned that it is getting colder and that ice sheets can form far more rapidly than was ever before realised. The long hot summer's days of the 1920s are gone for at least a few thousand years. Just around the corner are days like those remembered by Shakespeare, "when milk came frozen home in pail". Regular readers of Nature will already be aware of the more tragic consequences of that and the other climatic changes which are already becoming evident.

Given the topicality of the subject and the tremendous opportunity that it offered for some spectacular camera work the production was always just a little disappointing. Technically, of course, it came up to the usual excellent standard achieved by the BBC but in every other respect it was really rather dull television; those with black and white sets missed very little.

The production team could claim that spectacular entertainment was not their main objective in a programme which set out primarily to inform. That would be a fair argument but it is worth considering whether the programme fulfilled any requirements that had not been met already by other programmes; particularly so in view of the gigantic financial investment. Perhaps it is unfair to compare this production with Horizon, but the comparison is inevitable and, I believe, quite valid. Generally speaking, both series deal with the popularisation of serious science; several steps up from the antics of Patrick Moore and the boffinry of Raymond Baxter. And both attempt to keep the public abreast of recent advances in important areas of research.

Three Horizon programmes could have covered the same ground more effectively and perhaps more concisely. Quite definitely more cheaply. Hubert Lamb (who, as irrepressible as ever, made a brief appearance) and others like him will doubtless take that last point. How much better could those research workers have used the financial balance.

The Weather Machine had no inherent superiority over its more quotidian counterpart, only an ascribed importance supported by a cover picture in Radio Times. Although it was a brave attempt to present a comprehensive summary of the present state of a very wide field of research, perhaps the production should have gone out in two separate screenings. The real weather machine may well be changing gear; but in the present form a programme such as this can never really shift into top.

#### Book Review by John Gribbin

This is not at all a bad book. It covers a wide area, drawing on many disciplines, clearly, with lavish illustrations and at a very reasonable price. Even so, after reading it I was left with a slight feeling of disappointment because The Weather Machine does not quite live up to the standards of some of its predecessors (notably Violent Universe and Restless Earth) and it could have been better.

In spite of this, however, the book could well reach a wider audience than any of its predecessors. Climatic change, whether it 'merely' produces droughts in sub-tropical regions or leads to a full scale ice age, is rapidly becoming recognised as one of the key problems faced by mankind today. Changes in the weather are felt by

everyone, and they concern everyone in a way that advances in astronomical ideas or a better understanding of the causes of earthquakes can never do. Yet, because of the short time which has elapsed since it was realised just how quickly the climate can change, there are very few books which deal with the problem, although library shelves are laden with books on black holes, radio astronomy, earthquakes and continental drift written at all levels from the most popular to the most erudite.

When we turn to climatic change (as distinct from the weather and meteorology) I can think of only two books of broad interest that I would recommend: Hubert Lamb's Climate: Present, Past and Future (for the more academic market), and E. Le Roy Ladurie's Times of Feast, Times of Famine at a more popular level. Both are expensive, and neither provides a suitable starting point for the general reader who is concerned about what is happening to the weather.

So—partly by default—Nigel Calder's latest book is clearly the best introduction yet available to a concerned reader. As such, it will also prove invaluable to those studying these problems, although they will not all go along with the author in his firm support for the idea that ice ages are caused by the wobble of the Earth in its motion through space (the Milankovitch hypothesis). And no doubt hard core meteorologists will be interested in this concise summary of the longer view.

Perhaps the book could have gone a little further in presenting a balanced view rather than a picture of imminent climatic doom. Too much crying of "wolf" will do no good for the cause of the serious student of climatic change, and could indeed do harm. The reader is advised to take some of the extrapolations with a pinch of salt; but that is my only major criticism of what is, nevertheless, a worthwhile book.

There is still an obvious need for a more 'solid' book, as opposed to the coffee-table variety, to bridge the gap in the market between The Weather Machine and Lamb's epic-but until such a book appears this one will do to be going on with. As with most books one can quibble about points of presentation and style, but when the book has no competitors such criticism seems carping. One point, however: I would have liked to see the word 'climate' in the title; this is not really a book about weather, and the sub-title and The Threat of Ice gives, almost as a throwaway, a better impression of its theme.

The Weather Machine and The Threat of Ice. By Nigel Calder. Pp. 143. (BBC: London, November 1974.) £3.25.

# Purposeful drift of electrons

The Diffusion and Drift of Electrons in Gases. By L. G. H. Huxley and R. W. Crompton. Pp. xxiv+669. (Wiley Series in Plasma Physics.) (Wiley: New York and London, March 1974.) £19.60.

THE volume discusses the diffusion and drift of electrons in gases and little else. Dwelling only momentarily on any topics that may fringe the main theme. it concentrates without distraction on the high precision measurement of diffusion parameters and drift velocities primarily under low E/p conditions. Thus, for example, scant attention is afforded to transport phenomena under near breakdown conditions: the briefest of consideration is given to the Townsend coefficient; and ionisation and attachment are discussed with only moderate zeal. Indeed, the purpose of the measurements described is largely to provide an indirect and alternative technique for investigating low energy electron collision cross sections, yet even this topic is granted but one chapter at the end of the book and a more detailed and satisfactory account can be found elsewhere. In short then, this book is a powerful treatise on a very narrow subject.

The authors divide the book into two distinct parts dealing with theory and experiment separately. Most of the theory is developed from the Maxwell-Boltzmann equation and is concerned with the precise description of electron motion when a relatively small and constant electric field is present. Chapters are, however, devoted to high frequency fields and mixed electric and magnetic fields. A solitary chapter describing the alternative "free path" approach to the subject is a welcome addition to the section. The theory is developed rationally and the explanatory text is quite lucid. Thus, although the mathematical basis of this section may seem formidable to those not inclined in that direction (and most will find it a little tedious). it is an inevitable feature of the subject, and a more thorough or comprehensible alternative is probably not avail-

The experimental section is mainly devoted to the techniques used to measure the basic transport parameters and these are discussed at considerable—perhaps too great—length. Though in precision measurements the experimental detail often distinguishes between success and failure, no reasonable researcher will embark on a new project without having consulted the original publications. In a review—as this book is best described—it is adequate and probably advantageous to

be a little less meticulous. Nevertheless, the value and clarity of the account presented is beyond dispute. Rather inconspicuous chapters concerning the application of transport data to cross-section investigations and measurements of other, less fundamental, transport parameters conclude the section.

The book begins with an interesting historical introduction to the subject as a whole and ends with a useful summary of experimental data on the transport parameters and cross sections for a selected variety of gases. It is reasonably well referenced throughout. About one quarter of the references refer to original publications of the authors. The book will be unquestionably of value to those few with a direct interest in the subject.

R. M. Bull



An unstifled sneeze neatly captured, if not in a handkerchief, at least by high speed flash-illumination photography. From Biology: third edition. By John W. Kimball. Pp. xxiii+898. (Addison-Wesley: London, June 1974.) £5.35.

### Plants and pathogens

Genetics of Host-Parasite Interaction. By P. R. Day. Pp. xii+238. (Freeman: San Francisco and Reading, 1974.) £4.50.

In the first two editions of their book Fungal Genetics J. R. S. Fincham and P. R. Day included a chapter on the genetics of pathogenicity; they omitted it from the third edition (1973) stating that the subject had become too extensive for adequate treatment in a single chapter. Dr Day has now remedied this omission with a book which includes both hosts and pathogens.

Chapter 1 provides a short introduction to the subject. In the remainder of the book theoretical arguments and practical results are brought together to provide a comprehensive review of recent developments in ideas on the genetics of resistance (chapter 2) and pathogenicity (chapter 3), the gene-forgene concept (chapter 4), gene function in the host-parasite relationship (chapter 5), the genetical consequences of methods of disease control (chapter

6) and genetical aspects of parasite epidemics (chapter 7). The author describes his approach as speculative and at many points throughout the text gives alternative implications of theories and interpretations of results. These are always interesting and add greatly to the value of the book. The majority of examples are drawn from work on fungi but it is most useful to have many relevant references to work on other organisms. This is particularly the case in chapter 6 on biological and chemical control of plant disease where more experience has been obtained with insects than with other organisms.

The book contains over 600 references, mainly of recent origin: a most useful feature of the bibliography is that, after each reference, the numbers of the pages on which the work is quoted are given.

Use of the speculative approach makes chapter 5, on gene action, especially stimulating. In this rapidly developing area the contribution which genetic studies and the use of genetically controlled experimental material can make in helping to disentangle mere correlations from the true causes of disease resistance is emphasised. Theoretical models of gene action are described and their value in suggesting critical areas for study is illustrated. Similar value is attributed, in later chapters, to the production of computer programs for analysis of gene-forgene relationships, permitting the assignation of genes for resistance to related groups which can then be studied more intensively with conventional methods; it is also suggested that computer simulation of disease epidemics can help to identify factors for which insufficient data are available from practical investigations.

Similarities in the problems caused by genetic variability, common to attempts to control many parasites, are clearly demonstrated. In some cases, however, the application of a method of disease control may have consequences which are not confined solely to direct effects on genetic variability of the parasite. One might, for example, argue that, although destruction of barberry plants in North America did not, as Dr Day says in chapter 3, eliminate genetic variation in Puccinia graminis, it affected the overwintering behaviour of the pathogen, necessitating annual movements of spores between north and south, delaying the arrival of inoculum to northern areas in spring and changing genetic aspects of the epidemic; a fitting example for chapter 7 perhaps.

The book will appeal to advanced students and their teachers as well as to research workers and I welcome it as a timely and concise addition to the literature.

R. Johnson

# Advancing igneous petrology

The Alkaline Rocks. Edited by H. Sorensen. Pp. xii+622. (Wiley: London and New York, March 1974.) £20.00.

In this book Professor Henning Sørensen brings together contributions covering recent intensive investigations of the mineralogy, geochemistry and experimental behaviour of alkaline rocks, together with new information on their occurrence and field relations. The cover is wide but, deliberately, no attempt has been made to be comprehensive in terms of occurrence. Recently reviewed rock types (such as carbonatites) receive limited treatment, and the mineral parageneses of alkali pegmatites are not considered in detail.

In a short introduction Sørensen clarifies the complex terminology and definitions of the group and provides a brief historical review. The book is subsequently divided into six major sections concerned with petrography and petrology, regional distribution and tectonic relations, alkaline provinces, conditions of formation, petrogenesis, and economical (sic) geology. An appendix includes a useful glossary of over 400 alkaline and related rock names, and the book has been carefully indexed on the basis of rock names, subjects, geographical localities and authors.

In the first three major sections the contributions range from being largely descriptive to those with much petrogenetic content. It is difficult to do justice here to the wealth of ideas presented, but a number are of particular interest. The long duration of alkaline magmatism in certain areas is stressed by Sørensen in introducing the chapters on regional distribution. In this section, D. K. Bailey makes skilful use of African examples to examine the relationships between continental arching, rifting and magmatism, and concludes that volatile movement, with attendant heat and alkali transfer, may be important in the development of both flood and localised occurrences of alkaline rocks. The importance of structural controls over the location of magmatism in Siberia is stressed by E. L. Butakova, who also points out that in this region the development of alkaline rocks in stable areas seems to be linked with tectonic activity and calc-alkaline magmatism in adjacent fold belts. In contrast, M. Mathias does not find any obvious and simple correlation between alkaline rocks and structural features in southern Africa.

The alkaline provinces were selected to cover common rock associations, using well documented areas for which there were no readily available reviews. They are: the Kola Peninsula, South-

West Greenland, France and central Europe, the Mongol-Tuva province south-west of Lake Baikal, the Monteregian Hills in eastern Canada, Oceanic Islands and Niger-Nigeria. In several, the parental role of alkali olivine basalt or of melilite basalt is favoured, with extensive anorthosites requiring explanation in South-West Greenland and Niger. A. S. Pavlenko expresses a different viewpoint on the origins of the dominant alkali granites and miaskitic syenites of the Mongol-Tuva province. Here, the field and other evidence indicates a replacive origin for some at least of the alkaline rocks, which are seen as the climax of palingenesis of a thickened crust.

Reviewing • experimental studies, A. D. Edgar stresses the role of volatiles in alkali rock evolution (a topic also considered by L. N. Kogarko), discusses mechanisms by which thermal barriers may be crossed, and examines

# **Concepts for testing**

H. B. Barlow

Concepts and Mechanisms of Perception. By R. L. Gregory. Pp. xi+669. (Duckworth: London, September 1974.) £18.00.

This book consists of 56 articles by Richard Gregory and his collaborators. Some are only a page or two long, though the longest, and probably the most important, is a reprint of his study (with Jean Wallace) of the vision of a man who was blind from the age of a few months until he received a successful corneal graft at the age of 52. The book starts with a 30 page "Pretext" and short commentaries link and up-date each chapter. About 15 of the articles have not appeared before, but a good deal of this new material consists of descriptions of apparatus, patent specifications, or rather lightweight articles. The more substantial parts would be available to anyone with access to a good library, and it is not clear who will find it necessary or desirable to purchase this rather expensive collection.

Anyone who dips into the book will find that the articles and commentaries make light and easy reading; a delightful air of geniality pervades it. The "Pretext" especially is written with a constructive imagination that always makes it alive and interesting. But we know, since Popper and Fisher, that science advances by the brutal destruction of hypotheses, and I am not sure that the benign and gentle ideas that Gregory advances will survive for long in the vicious jungle of facts uncovered by critical psychologists.

the conditions of formation of specific minerals. Researches on homogenisation temperatures of inclusions in minerals, described by V. S. Sobolev and others, indicate temperatures in excess of 850°C during nepheline syenite crystallisation, whereas phenocrysts in effusive rocks may have commenced crystallisation at appreciably higher temperatures (>1,250°C).

Several of the contributors dealing with petrogenesis express doubts on any simple parental role of alkali olivine basalt in alkali rock genesis, although this is clearly a reasonable assumption in Oceanic Islands and in provinces such as France and central Europe, and South-West Greenland. J. L. Powell and K. Bell find that the isotopic composition of strontium may indicate derivation from continental basalt for many alkaline rocks but processes involving partial fusion of inhomogeneous source material may also be involved, as may assimilation, although not of limestone (a process also not favoured by P. J. Wyllie in a later chapter). D. K. Bailey argues strongly in favour of derivation of felsic alkaline magmas by partial melting in the deep crust. After reading the petrogenesis section one is left with the impression that relatively little is known about the nature and structure of the supposed source areas for the alkaline magmas in the upper mantle or the deep crust. It is to be hoped that this imbalance will be corrected in an extension of geophysical investigations, of the type in progress in East Africa and the Oslo Fjord area, to other alkaline provinces, and that these will be integrated with the petrological researches.

The common development of layering and other features of igneous cumulates indicate the importance of crystal fractionation in alkali rock formation, a topic given detailed consideration by R. Macdonald. The sequence of types in many alkali associations is, however, not always readily explicable in terms of crystal fractionation of a parent magma, and the suggestion by L. N. Kogarko and others that compositionally graded magma chambers may form from volatile migration along P-T gradients is welcome and deserves to be explored further.

The book contains a wide range of informative and stimulating contributions. Professor Sørensen is particularly to be congratulated on obtaining, with the help of Dr V. P. Volkov, contributions from so many Russian petrologists. The book is well produced and clearly illustrated with numerous line diagrams. It is a volume which can be thoroughly recommended to everyone interested in advanced igneous petrology.

C. H. Emeleus

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#### DIRECTOR

Applications are invited for the position of DIREKTOR DES INSTITUTS FÜR TIERZUCHT UND TIERVERHALTEN FORSCHUNGSANSTALT FÜR LANDWIRTSCHAFT.

LANDWIRISCHAFI,

a federal research institution engaged in reproductive biology, animal behaviour and enetics. Staff of 250 including 16 scientists. Research budgeted. Facilities: 2,500 m² of laboratory space; 4 research farms (1200 ha); live stock of 1,200 cattle, 2,000 pigs, 1,000 sheep. Locations: main laboratories and 2 farms at Mariensee near Hannover; laboratory and 2 farms at Trenthorst near Lübeck.

Qualifications: Internationally established research reputation. Applicants with biochemical and physiological background should be enabled to coordinate the research programmes of the different sections.

coordinate the research programmes of the sections.
Salary: approx. 65,000 DM/annum (B 3 tenure position in government service).
To apply, send curriculum vitae, summary of research activities, list of publications and reprints before December 31, 1975 to:
Prof. Dr E. Zimmer
Präsident of Forschungsanstalt für Landwirtschaft Bundesallee 50
D 33 Braunschweig
Federal Republic of Germany
(1792)

#### THE HORMEL INSTITUTE OF THE UNIVERSITY OF MINNESOTA

UNIVERSITY OF MINNESOTA invites applications from qualified individuals for the position of EXECUTIVE DIRECTOR of The Hormel Institute which is located in Austin, Minnesota. The Executive Director is responsible for overall administration of an institute with 80-90 employees, including 20 of whom have academic rank. Candidates for this position should be recognised authorities in the lipid field, and should have at least 12 years of research experience in the field. The director is expected to maintain an active research programme.

Closing date for receipt of all materials is February 1, 1975. Send curriculum vitae, a list of publications and names of three referees to Dr J. E. Gander (Chairman, Search Committee), Department of Biochemistry, University of Minnesota, St Paul, MN 55108. (1886)

#### **BIOCHEMICAL TECHNICIAN**

Biochemical Technician required for small laboratory engaged in Metabolic Studies. Some experience with enzymes an advantage. Salary £1,800 to £2,800. Please apply with details of experience and names and addresses of two referees to: The addresses of two referees to: The Director, Alfred Chester Beatty Body Dynamics Laboratory, Brooksden, Cranbrook, Kent TN17 3DT.

(1897)

## **Senior Pharmacologist Anti-Inflammatory Research**

Applications are invited for a senior post in our Pharmacology Department in Welwyn Garden City, Hertfordshire. The department is responsible for carrying our research and screening work aimed at the development of new drugs in which the pharmacologists participate in the research programmes of interdisciplinary groups.

The senior pharmacologist we appoint will be responsible for directing the work of a team of pharmacologists engaged in anti-inflammatory research. Applicants should have a Ph.D. qualification and preferably some post-doctoral experience in anti-inflammatory research.

Our Pharmacology Department is located in new laboratories where the facilities and working conditions are excellent. Conditions of service are above average and in appropriate cases generous assistance with relocation will be available.

Roche Products Limited is part of a major international pharmaceutical firm based in Switzerland and is itself one of the leaders in the industry in the United Kingdom. If you would like to apply for this post please write for further information, and a Company booklet, to the Welwyn Personnel Manager.

Closing date for applications, December 13, 1974.



Roche Products Limited Welwyn Garden City Hertfordshire AL7 3AY

(1896)

#### INSTITUT MAX VON LAUE-PAUL LANGEVIN GRENOBLE - FRANCE

The Institut Max Von Laue-Paul Langevin operates a high flux reactor providing intense beams of neutrons for studies of condensed matter in the fields of physics, chemistry, biology and materials science. Visitors from universities and research centres in the member countries, France, Germany and the U.K., and resident scientists use the high flux beam reactor and the Institut provides scientific and technical support.

Applications are invited for the following posts at Grenoble:

#### THEORETICAL PHYSICISTS

Vacancies exist at the Institut for theoretical physicists interested in the microscopic properties of condensed matter. Successful applicants would join a theoretical physics group which complements the wide range of research studies that are conducted with neutron scattering techniques. Candidates should possess an appropriate degree and preferably have had post-doctoral experience in solid state, liquid or polymer physics.

The appointment would be for two years in the first instance with a possibility of an extension.

Salary will be according to qualifications, experience and responsibilities. Removal expenses will also be paid and assistance given in seeking accommodation.

Write for application form to: Mr D. McConville, Science Research buncil, c/o British Rail Engineering Ltd, Swindon Works, Swindon. Council, c/o British Rail Engineering Ltd, Swindon Wo Wiltshire, SN1 5BW, quoting reference and post applied for.

Completed application form should be returned by December 16, 1974. Ref: ILL/01. (1945)

#### INSTITUT MAX VON LAUE-PAUL LANGEVIN **GRENOBLE — FRANCE**

The Institut Max Von Laue—Paul Langevin operates a high flux reactor providing intense beams of neutrons for studies of condensed matter in the fields of physics, chemistry, biology and materials science. Visitors from universities and research centres in the member countries, France, Germany and the U.K., and resident scientists use the high flux beam reactor and the Institut provides scientific and technical support.

Applications are invited for the following post at Grenoble:

#### **ENGINEER-**SAMPLE ENVIRONMENT SECTION

The successful applicant will work in a team and be responsible to the Section Head for the design, acceptance tests and routine operation of special equipment (cryogenics, high pressure, high temperature) used for the control of the environment of specimens studied in the instruments of the

high flux reactor.

Applicants should possess a degree or equivalent qualification and have had some years' experience in a research laboratory. In particular they should have a good practical and theoretical knowledge of cryogenics (several years experience) and a practical knowledge of vacuum technology. A knowledge of neutron scattering physics would be an advantage. A working knowledge of French is desirable but not immediately essential as tuition will be given.

Salary will be according to qualifications, experience and responsibilities. Removal expenses will also be paid and assistance given in seeking accom-

modation.

Write for application form to: Mr D. McConville, Science Research Council, c/o British Rail Engineering Ltd, Swindon Works, Swindon, Wiltshire, SN1 5BW, quoting reference and post applied for.

Completed application form should be returned by December 16, 1974.

Ref: ILL/02 (1946)

# PLANT VIROLOGIST

£2,461 to £4,441

Applications are invited for a permanent and pensionable appointment in the Plant Pathology Division of the Department of Agriculture.

The duties involve conducting research on plant virus diseases with particular reference to diseases of grass, cereal and horticultural crops.

The successful applicant may be required to undertake teaching duties in the Department of Mycology and Plant Pathology, Faculty of Agriculture and Food Science, The Queen's University, Belfast.

The appointment may be at Senior Scientific Officer or Higher Scientific Officer level.

Senior Scientific Officer should be over 25 and under 32 years of age with a • first or second class Honours degree in a scientific or agricultural discipline pertinent to Plant Pathology and at least four years post-graduate experience in Plant Virology.

Higher Scientific Officer should be under 30 years of age with an Honours degree as above and at least two years relevant post-graduate experience.

Salary scales: Senior Scientific Officer - £3,157 to £4,441 Higher Scientific Officer - £2,461 to £3,371

Grading and starting salary will be related to qualifications and experience. A cost of living supplement is also payable.

lease write or telephone for an application form, quoting Ref. SB 308/74/N, to Civil Service Commission, Clarendon House, Adelaide Street, Belfast BT2 8ND (telephone 0232 44300, ext. 26). Completed forms must be returned to arrive not later than December 5, 1974. (1965)



#### PENNSYLVANIA STATE UNIVERSITY

DEPARTMENT OF PHYSICS

DEPARTMENT OF PHYSICS

Assistantships are available for graduate students wishing to pursue a Ph.D. program, starting September 3, 1975. Major research areas: Solid state and surface physics, field ion microscopy, fow temperature physics, molecular spectroscopy, laser physics and nonlinear optics, vacuum and ionosphere research, acoustics, particle and Q.E.D. theory. Half-time teaching assistantships carry a stipend of \$3,852 for nine months. Quartertime summer teaching appointments and some all-year research appointments are available. Address inquiries to Prof. R. H. Good, Jr., Head, 104 Davey Laboratory, University Park, Pa., U.S.A. 16802. (1770)

#### NATIONAL COUNCIL FOR SCIENTIFIC RESEARCH

Applications are invited from suitably qualified persons for the post of

#### TIMBER RESEARCH OFFICER

Applicants must have a degree or equivalent pro-fessional qualification in timber or structural engineering and experience in the use of timber in the mining and other industries.

The timber research officer's duties will include The timber research officer's duties will include making surveys of timber and forest products utilization by the Zambian industries including the mining industry, planning and executing applied research in the use of timber and forest products and liaising with industries on timber and forest products utilization.

Salary according to qualifications and experience on the scales:—
Senior Professional Officer

K4,680 by 240 to K5,640

Principal Professional Officer

K4,680 by 240 to K5,640
Principal Professional Officer
K5,840 by 240 to K6,800
Senior Principal Professional Officer
K7,000 by 200 to K7,600
There is a superannuation scheme for Zambians.
Non-Zambians will be paid a graduated gratuity of 20% of the total earnings in the first year. 25% in the second year and 30% in the third year of resident service. Passages will be paid for the officer, wife and minor dependent children.

while and minor dependent children.

Applications giving full personal details, qualifications, experience and naming three referees should be submitted to:—

The Secretary General,
National Council for Scientific Research,
P.O. Box CH 158,
Chelston, Lusaka,
Zambia. (1794)

#### UNIVERSITY COLLEGE LONDON DEPARTMENT OF BIOPHYSICS ELECTRON-MICROSCOPIST RESEARCH ASSISTANT

required to work with Professor R. Miledi on problems in Neurobiology, which forms part of a long-term Project supported by the M.R.C.

Preference given to applicants with experience of freeze-etching or analytical electron-microscopy. Salary within Lecturer scale plus London Allow-

Applications with curriculum vitae and names and addresses of two referees to Mrs K. Garnons-Williams, Biophysics Dept (N), University College London, Gower Street, London WC1E 6BT. (1883)

#### CLINICAL MICROBIOLOGIST (Ph.D.) CANADA

The Hamilton District Programme in Laboratory Medicine requires a Clinical Microbiologist to be located at the Hamilton General Hospital. The appointee should possess a Ph.D. in clinical microbiology. Training and experience in Mycology would be an advantage. The appointment entails responsibilities in the supervision of the clinical laboratory and teaching in the Microbiology/Infectious Disease Educational Programmes of the Faculty of Health Sciences, McMaster University and the District Programme in Laboratory Medicine. Facilities for research are available. The position carries an appointment in the Department of Pathology, McMaster University, appropriate to qualifications and experience.

Applications and experience.

Applications, enclosing curriculum vitae, transcript, and the names of three referees, to:—
Dr. I. O. Stewart,
Head of Microbiology, and District
Co-ordinator for Microbiology/Infectious Disease,
Hamilton General Hospital,
Hamilton, L8L 2X2,
Ontario, Canada Hamilton, L8L 2 Ontario, Canada.

(1954)

#### ASSISTANT PROFESSORS (Biological Sciences)

(Biological Sciences)

Several positions are open for biologists with promise of creative and dynamic research programs in plant tissue culture, immunobiology, developmental biology, biochemical taxonomy, neurobiology, behavioral or human genetics. Share in teaching large elementary course, or pre-professional courses (embryology, animal physiology), and develop advanced courses in areas of speciality. Send resume and references to: A. Eisenstark, Director, Division of Biological Sciences, University of Missouri, Columbia, Missouri 65201 U.S.A. University of Missouri of Missouri is an affirmative action, equal opportunity employer. (1953)

#### TERRESTRIAL PLANT ECOLOGIST/SOCIOLOGIST

Dalhousie University has a vacancy from July 1, 1975 for a plant ecologist/sociologist. Qualifications: Ph.D. and proven ability in teaching and research. Duties include teaching at the undergraduate and graduate levels, and developing a vigorous research program in terrestrial plant ecology. There is a possibility for major research support in interdisciplinary forest studies. Salary and rank negotiable; a senior appointment is possible. Curriculum vitae and names of three referees to be sent to: Chairman, Department of Biology, Dalhousie University, Halfax, N.S. Canada B3H 4J1. Closing date: when position filled. (1955)

#### YORK UNIVERSITY DEPARTMENT OF CHEMISTRY

DEPARTMENT OF CHEMISTRY

Applications are invited for a senior position available July 1, 1975, in the field of chemical physics with experience in laser photochemistry. The successful candidate will play a leading role in establishing a major research centre in this field. The appointment will be made at the rank of Full or Associate Professor. Salary negotiable and commensurate with rank. Send curriculum vitae, reprints and names of three referees to Professor G. O. Aspinall, Department of Chemistry, York University, Downsview, Toronto, Ontario M3J 1P3, Canada. (1957)

#### UNIVERSITY OF BERNE DEPARTMENT OF PAEDIATRICS

Position vacant for RESEARCH BIOCHEMIST in Switzerland. Required: Experience in modern separation techniques for proteins and glycopro-

teins.

Salary according to experience. Team-work in a Medical Research Unit.

Applications should be sent to: Professor B. Hadorn
Gastrointestinal Unit

University Children's Hospital Freiburgstrasse 23

3010 BERNE (Switzerland).

(1958)

#### UNIVERSITY OF ILLINOIS AT **URBANA-CHAMPAIGN** PLANT BIOPHYSICIST. ASSISTANT PROFESSOR

for Fall 1975. Joint appointment in Departments of Physiology & Biophysics (Biophysics Division) and Botany. Postdoctoral research experience, publications essential. Teaching includes developing a plant biophysics course. Apply to: Dr. Thomas G. Ebrey, 524 Burrill Hall, University of Illinois, Urbana, Illinois 61801. The University of Illinois at Urbana-Champaign is an Affirmative Action/Equal Opportunity Employer and encourages applications from members of minority groups and women. (1959)

#### WITHINGTON HOSPITAL

#### MANCHESTER M20 8LR

The National Reference Laboratory for Anticoagulant Control Reagents requires a MEDICAL LABORATORY TECHNICIAN. A.I.M.L.T. qualification or good honours degree in applied science necessary. Appointee will be trained in basic routine blood coagulation procedure and will participate in routine work, research and development. Good career prospects.

Applications in writing naming two referees to the Hospital Secretary, quoting reference B46. (1978)

#### INSTITUT MAX VON LAUE-PAUL LANGEVIN GRENOBLE - FRANCE

The Institut Max Von Laue-Paul Langevin operates a high flux reactor providing intense beams of neutrons for studies of condensed matter in the fields of physics, chemistry, biology and materials science. Visitors from universities and research centres in the member countries. France, Germany and the U.K., and resident scientists use the high flux beam reactor and the Institut provides scientific and technical support.

Applications are invited for the following post at Grenoble:

#### TECHNICIANS FOR THE NEUTRON BEAM EXPERIMENTS

The Institut is currently forming a small technical group within the general service section with a view to ensuring the maximum exploitation of instruments. The group is expected to collaborate with engineers on the design of new neutron beam experimental equipment, assist with its installation and testing and collaborate with the physicists and technicians on the established instruments when required.

Applicants should possess O.N.C. or equivalent qualifications and have had research laboratory experience in some of the following areas: high vacuum, high temperatures, high pressures, cryogenics, mechanical and electrical engineering. A knowledge of French is desirable but not essential.

Salary will be according to qualifications, experience and responsibilities. Removal expenses will also be paid and assistance given in seeking accommodation.

Write for application form to: Mr D. McConville, Science Research Council, c/o British Rail Engineering Ltd, Swindon Works, Swindon, Wiltshire, SN1 5BW, quoting reference and post applied for.

Completed application form should be returned by December 16, 1974. Ref: ILL/03



# Neuropharmacologist

We would like to appoint a Pharmacologist with post doctoral research experience to a position in our CNS unit. The successful candidate will have a thorough knowledge of laboratory methods for the detection and evaluation of compounds which interact with the central nervous system and will be keen to generate and test new ideas in collaboration with chemists, biochemists and clinicians.

This appointment could be attractive to a scientist who has held a post in a university or in industry.

Conditions of service, prospects and assistance given in moving home, are designed to attract and retain staff of this high calibre and would be discussed in detail at interview.

Applications in writing, giving brief details of age, experience and qualifications, should be sent to:

M. F. Losse, Personnel Officer Imperial Chemical Industries Limited Pharmaceuticals Division Mereside, Alderley Park Nr. Macclesfield, Cheshire.

(1977)

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# SCOTTISH SOCIETY FOR RESEARCH IN PLANT BREEDING CYTOLOGIST

Applications are invited for the post of Scientific Officer/Higher Scientific Officer in the Cytology Laboratory of the Scottish Plant Breeding Station. The person appointed will be required to take charge of the Laboratory, which provides a cytology service to breeders working on cereals, forage grasses and potatoes.

The Scottish Plant Breeding Station is financed by the Department of Agriculture and Fisheries for Scotland under scientific advice from the Agricultural Research Council and is a component of the Agricultural Research Service within which common conditions of service apply. It is located about 1½ miles south of Edinburgh City boundary, and comprises modern laboratories, glasshouses, land and other facilities.

Qualifications: Minimum, H.N.C. pass degree or equivalent in Botany, agricultural Botany, Plant Science or Biology and preferably with training or experience in general microscopy and chromosome studies. At least five years' appropriate scientific experience are required for appointment to Higher Scientific Officer.

Salary: depending on qualifications and experience. Scientific Officer—£1,592 to £2,675 Higher Scientific Officer—£2,461 to £3,371. The post is superannuable.

Application forms from the Secretary, Scottish Plant Breeding Station, Pentlandfield, Roslin, Midlothian, EH25 9RF to be returned not later than December 6, 1974.

Applicants from the Agricultural Research Service should submit their application through their present Director if they wish to be considered for the award of "public interest" transfer terms. (1962)

# MEDICAL RESEARCH COUNCIL NATIONAL INSTITUTE FOR MEDICAL RESEARCH DIVISION OF CYTOPATHOLOGY CELL BIOLOGIST OR ELECTRON MICROSCOPIST

ELECTRON MICROSCOPIST

Applications are invited from young postdoctoral scientists (up to the age of 27 years) for
a position with a group investigating the fine
structure and properties of cellular membranes,
particularly in relation to virus infections. The
successful applicant will be involved in biological
preparative methods; experience in immunolabelling, auto-radiography, thin-sectioning, or
cryo-techniques for electron microscopy would
therefore be an advantage. The appointment
will be for up to three years and will be in the
salary range £2,412 to £3,636 p.a. according to
age and qualifications (plus London and threshold
allowances). Superannuation provision is under
F.S.S.U.

Applications giving details of qualifications. ex-

F.S.S.U.

Applications giving details of qualifications, experience and the names of two professional referees, should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA before December 14, 1974. (1960)

# PHYSICAL CHEMIST

#### (M.Sc. or Ph.D.)

We have a vacancy in our new Research and Development Centre at Paisley for a young physical chemist, preferably with some experience of colloid chemistry.

The successful candidate will join a small research team actively engaged in studies of the physico-chemical properties of organic pigments. He will be concerned primarily with the properties of dispersions in liquid media. There is great scope for original thought and enthusiasm in this vital position. It is essential that we continue to improve our knowledge of the underlying mechanisms which control this young and growing technology.

Our laboratories are well equipped to make appropriate physicochemical and rheological measurements and excellent back-up analytical facilities are available, including electron microscopy and X-ray diffraction. Complementary surface chemical studies are in progress using gas/ vapour adsorption and calorimetric techniques.

We anticipate that suitable applicants will possess at least an M.Sc. degree, but preferably a Ph.D., and will be aged around 25/30 years.

An attractive starting salary will reflect the importance of this position, and there is a substantial range of fringe benefits. Generous assistance will be given with removal and relocation expenses where applicable.

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Please write with brief details of qualifications, research experience and present position to:—

Mr. D. Glenn,
Senior Personnel Officer,
CIBA-GEIGY (UK) Limited,
Pigments Division,
Hawkhead Road,
Paisley PA2 7BG.



1974

# CIBA-GEIGY

(1948)

#### UNIVERSITY OF NATAL DEPARTMENT OF GEOLOGY PIETERMARITZBURG

Applications are invited from suitably qualified persons for appointment to the post of

#### LECTURER IN STRUCTURAL GEOLOGY, MINERALOGY AND CRYSTALLOGRAPHY

The salary scales attached to the post are: R4,800 by 300 to R6,900 plus 15% pensionable allowance. A general upward revision of salary scales is expected immediately.

The commencing salary notch will be dependent on the qualifications and/or experience of the successful applicant. In addition, an annual vacation savings bonus is payable, subject to Treasury regulations.

Treasury regulations,

Application forms, further particulars of the post and information on pension, medical aid, staff bursary, housing loan and subsidy schemes, long leave conditions and travelling expenses on first appointment are obtainable from the Registrar, University of Natal, King George V Avenue, Durban, South Africa, with whom applications, on the prescribed form, must be lodged not later than January 17, 1975, quoting reference Adv. 139/74. (1969)

#### ROYAL HOLLOWAY COLLEGE (UNIVERSITY OF LONDON) Egham Hill, Egham, Surrey.

#### EXPERIENCED ELECTRONICS TECHNICIAN (GRADE 4)

Required in the Physics Department for 1 year only. Salary in the scale £1,848 to £2,163. Applications together with the names and addresses of two referees should be sent to the Personnel Officer (N) as soon as possible. (1970)

#### UNIVERSITY OF READING DEPARTMENT OF PHYSIOLOGY & BIOCHEMISTRY

#### RESEARCH DEMONSTRATOR

RESEARCH DEMONSTRATOR
required immediately to carry out research for a higher degree in any one of the following subjects: steroid biochemistry of the mature and immature mammalian testis and epididymis; glutamate and glutamine metabolism in the foetal and neonatal sheep; fatty acid synthesis in the rabbit mammary gland; organic constituents of egg shells; protein and amino acid metabolism in the growing rabbit; determination of the primary lesion of heat damage in the testis; physiological studies of the avian oviduct; study of the nitrogen metabolism in the coprophagous mammal. Salary £1,122 by 54 to £1,230 p.a. plus threshold supplement. Candidates should have a good second class Honours degree in a relevant subject. The post will be for three years with the possibility of extension; about 12 hours demonstrating per week during term time. Enquiries and/or applications, with the names of 2 academic referees, should be addressed to Professor G. M. H. Waites, Department of Physiology and Biochemistry, The University, Whiteknights, Reading RG6 2AJ. Telephone Reading 85123 ext. 7675. (Ref. TN 95). Closing date: December 16, 1974. (1971)

#### DANISH SPACE RESEARCH INSTITUTE **COPENHAGEN**

invites applications for a research position in the cosmic ray group. Ph.D. degree is required.

The main interests of the group lie in the study of the isotopic composition of galactic cosmic radiation.

Applicants should have a proved competence and a strong interest in the development of new experimental techniques. Some experience in initiating and directing research programmes would be useful.

Salaries for research workers in Denmark are approximately equivalent to those prevailing in the U.K. They follow rules set by government and depend on qualifications.

Appointment will be for one year with possibility of renewal.

possibility of renewal.

Interested persons are asked to send a resume of their experience and references to:

B. Peters, Director

Danish Space Research Institute

Lundtoftevej 7

2800 Lyngby, Denmark (1975)

#### NOTTINGHAM AREA GENETIC SERVICE Maternity Unit and City Hospital

# **BASIC GRADE** SCIENTIFIC OFFICER

Applicants should possess a degree in Biology and preferably have a background of genetics. The post will involve diagnostic analysis of human chromosomes from long and short-term tissue cultures.

Salary scale: £2,046 to £2,562 for 1st and 2nd class Honours degrees; £1,689 to £2,562 for ordinary degrees.

Further information can be obtained from Dr Cooke, Tel: 68111 Ext. 2785.

Application forms from the Personnel Department, Sherwood Hospital, Hucknall Road, Nottingham. Tel: 600459 any time.

(1961)

#### Applications are invited from graduates in chemistry for

#### FREE-LANCE EDITORIAL WORK

on manuscripts and proofs, Essential requirements are English as mother-tongue, an appreciation of good English style, and a meticulous eye for detail. Applications, in writing, with details of experience and qualifications (including specialist field of study) should be addressed to Miss T. Sijpesteyn, Elsevier Scientific Publishing Company, P.O. Box 330, Amsterdam, The Netherlands.

#### ROYAL HOSPITAL FOR SICK CHILDREN EDINBURGH

#### CYTOGENETICIST

There is a vacancy for a graduate within the cytogenetic unit of this hospital. The unit provides a diagnostic cytogenetic service for a number of hospitals and is responsible for the amniotic fluid cell culture for the Lothians area.

Experience in cytogenetics, and preferably tissue culture, is essential. Opportunities for a higher degree may be available.

Salary within the range of £1,497 to £2,694 depending on qualifications and experience.

Applications stating experience and the names and addresses of two referees to the Sector Administrator, Royal Hospital for Sick Children, 1 Rillbank Terrace, Edinburgh EH9 1LN.

# ■ Lothian Health Board

#### SCOTTISH SOCIETY FOR RESEARCH IN PLANT BREEDING

#### POTATO BREEDER/NEMATOLOGIST

Applications are invited for the post of Scientific Officer/Higher Scientific Officer in the Potato Department of the Scottish Plant Breeding Station to work on (a) breeding projects for the transfer of genetic resistance to potato cyst eelworm to cultivated potatoes and (b) studies of resistance, pathogenicity and host-parasite relations.

The Scottish Plant Breeding Station is financed by the Department of Agriculture and Fisheries for Scotland ander scientific advice from the Agricultural Research Council and is a component of the Agricultural Research Service within which common conditions of service apply. It is situated about 1‡ miles south of Edinburgh City boundary, and comprises modern laboratories, glassshouses, land and other facilities.

Qualifications: 1st or Upper 2nd class honours degree in Plant Science, Zoology, Biology or Genetics. At least two years' postgraduate research, preferably in genetics or nematology, are required for appointment to a Higher Scientific Officer.

Salary: depending on qualifications and experience. Scientific Officer—£1,592 to £2,675 Higher Scientific Officer—£2,461 to £3,371. The post is superannuable.

Application forms from the Secretary, Scottish Plant Breeding Station, Pentlandfield, Roslin, Midlothian, EH25 9RF to be returned not later than December 6, 1974.

Applicants from the Agricultural Research Service should submit their application through their present Director if they wish to be considered for the award of "public interest" transfer terms. (1963)

# HIGHER SCIENTIFIC OFFICER £2,461 to £3,371

Applications are invited for the post of Higher Scientific Officer in the Field Botany Research Division of the Department of Agriculture.

The duties will include responsibility for seed certification and seed testing.

Applicants must be under 30 years of age on December 31, 1974 and have a degree in Agriculture or an appropriate Agricultural Science; or an H.N.D. in Agriculture; or an equivalent qualification acceptable to the Civil Service Commissioners. Some knowledge and experience of seed technology is essential.

Starting salary will be related to qualifications and experience. A cost of living supplement is also payable. There are prospects of promotion to Senior Scientific Officer (£3,157 to £4,441).

Please write or telephone for an application form, quoting Ref. SB 309/74/N, to Civil Service Commission, Clarendon House, Adelaide Street, Belfast BT2 8ND (telephone 0232 44300, ext. 26). Completed forms must be returned to arrive not later than December 5, 1974. (1967)



#### ROYAL VICTORIA HOSPITAL DEPARTMENT OF MICROBIOLOGY PRINCIPAL SCIENTIFIC OFFICER

Applications are invited from those holding a degree in a biological science. The person required must be widely experienced in Microbiology including automation and microbiological assay techniques and be able to evaluate new methods, including quality control in relation to diagnostic bacteriology and secology.

bacteriology and serology.

A candidate with less experience may be given an interim appointment as a Senior Scientific Officer.

#### SALARY SCALE: £4,482 to £5,796 per annum.

Application forms may be obtained from the District Personnel Department, Eastern Health and Social Serwices Board, North and West Belfast District, Royal Victoria Hospital, Grosvenor Road, Belfast BT12 6BA, to be returned by Friday, December 13, 1974. (2003)

#### HISTOLOGY TECHNICIAN

required for Pharmacological and Toxicological Laboratory. Experience in the preparation and processing of animal tissues essential. Good working conditions, Pension and Assurance Scheme. Application forms from: The Secretary, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London N1 2HB. (1996)

# UNIVERSITY OF READING WOLFSON RESEARCH PROJECTS

Applications are invited for research officers—salaries in the range £2,000 to £4,000 per annum—for two projects supported for a four year period by grants from the Wolfson Foundation for the development of U.K. resources, viz:—(i) Oilseed utilisation—edible oil and protein; (ii) leaf protein for human use. Candidates should have degrees in a suitable subject, such as chemistry, biochemistry, or chemical engineering, research experience and preferably some industrial experience. There are vacancies also for graduate or non-graduate research assistants.

Applicants should send a detailed statement

Applicants should send a detailed statement of career together with a short covering letter and the names of two referees to Professor R. Aylward, Department of Food Science, University of Reading, London Road, Reading RGI 5AQ. (Ref: MN 55). (1972)

#### UNIVERSITY OF KENT AT CANTERBURY RESEARCH ASSISTANT IN BIOCHEMISTRY

Applications are invited for the post of Research Assistant in Biochemistry to work on aspects of the control of amino-acid and carbohydrate metabolism by hormones and other effectors in in vitro systems under the supervision of Dr C. I. Pogson. Applicants should have a degree in Biochemistry or a related subject.

chemistry or a related subject.

The appointment, which is supported by the Medical Research Council, will be for two years on the scale £1,317 to £1,782 (Eligible for threshold payments). Application forms and particulars may be obtained from the Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent CT2 7NH. Please quote ref. A74/74. Closing date for applications December 6, 1974. (1979)

### THE HANNAH RESEARCH INSTITUTE

#### HIGHER SCIENTIFIC OFFICER

required to assist with work on the metabolism of polyunsaturated fatty acids by ruminant animals. Candidates should have a degree or H.N.C. in Chemistry or Biochemistry with at least 5 years' appropriate post qualifying experience.

Starting salary in the range £2,461 to £3,371 plus Threshold Agreement according to qualifications and experience. The post is pensionable.

Applications giving age, curriculum vitae and the names of three referees should be forwarded to The Secretary, The Hannah Research Institute, AYR KA6 5HL by December 10, 1974. (1980)

# UNIVERSITY OF LEICESTER DEPARTMENT OF GENETICS (Head of Department: Professor R. H. Pritchard)

Applications are invited for a post of

#### LECTURER

The person appointed will participate in the teaching of genetics to both medical students and those studying for the B.Sc. in Biological Sciences. Candidates must have a broad knowledge of genetics and preference will be given to those who combine this with postdoctoral experience in some aspect of nucleic acid technology.

Initial salary according to qualifications and experience within the range £2,118 to £4,896 a year plus threshold payments, at present £167.04, plus Superannuation. Further particulars from the Registrar, to whom applications should be sent quoting reference NLG by January 5, 1975.

(1986)

#### ST THOMAS'S HOSPITAL MEDICAL SCHOOL (University of London)

#### London SE1 7EH RESEARCH ASSISTANT

required for three year Virology/Immunology project. Suitable for PhD candidate.

Written applications to Dr J. E. Banatvala from whom further particulars may be obtained.

#### THE UNIVERSITY OF **MANCHESTER**

KEEPER OF ZOOLOGY

Applications invited for the above post in the MANCHESTER MUSEUM. Duties to commence on October 1, 1975. Candidates should be graduates in Zoology and should have experience in curating zoological collections, the most important of which in the department belong to mollusca and birds. The Diploma of the Museums Association would be an advantage. Salary range £2,580 to £4,896 p.a. F.S.S.U. Further particulars and application forms (returnable by January 1, 1975) from the Registrar, The University, Manchester, M13 9PL. Quote ref: 239/74/N. (1987)

#### UNIVERSITY OF CAMBRIDGE DEPARTMENT OF APPLIED BIOLOGY

#### FIELD STATION SUPERINTENDENT

Vacancy exists for an Assistant Technical Officer to be superintendent of the Department's Applied Biology Field Station. Candidates should have had experience of experimental field station and glasshouse operations and preferably possess an appropriate qualification. Tenure three years first instance; extendable. Pensionable salary in scale £1,941 to £3,108 (7 increments). Starting point determined by age, qualifications and experience. Superannuation with F.S.S.U. Assistance with removal expenses. Further details from and applications in writing giving full particulars of qualifications and experience and names and addresses of not more than three referees to the Secretary, Faculty Board of Biology 'A', Department of Applied Biology, Downing Street, Cambridge, CB2 3DX, not later than December 20, 1974. (1991)

#### THE UNIVERSITY OF SHEFFIELD DEPARTMENT OF CHILD HEALTH RESEARCH ASSISTANT

Applications are invited for the above post from science graduates. Work in the Department includes clinical and animal studies on development in the infant of renal and respiratory control and the study of the effect of anti-tumour agents on growth. Salary range £1,905 to £2,247. Further particulars from: The Registrar and Secretary, The University, Sheffield \$10 2TN, to whom applications should be sent by December 6, 1974. Quote Ref. 151/G. (1992)

#### PUBLIC HEALTH LABORATORY SERVICE

#### BACTERIAL METABOLISM RESEARCH LABORATORY

Applications are invited from biochemists/
chemists with a Ph.D. and an interest in
bacteriology for work on the identification of
minor faecal bile acids as part of a study of the
role of bacteria in the causation of intestinal cancer. The laboratory is located at Colindale and is
well equipped. The appointment is for a limited
period. N.H.S. salary scales and terms and conditions of service. Applications to: Professor R. E. O.
Williams, Public Health Laboratory Service Board,
Lower Entrance, Colindale Hospital, Colindale
Avenue, London NW9 5FO. Lower Entrance, Colindale Avenue, London NW9 5EQ. Hospital, Colindale (1994)

#### THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

(University of London) DEPARTMENT OF IMMUNOLOGY

Applications are invited for a postdoctoral research assistant to work on the immune response of mice to parasites. Salary up to £2,580 per annum plus London allowance and superannuation under F.S.S.U.

Applications including curriculum vitae and the names and addresses of two referees should be submitted to Professor I. M. Roitt, Department of Immunology, Arthur Stanley House, 40-50 Totenham Street, London WIP 9PG by December 15, 1074 (1995)

# SCIENTIFIC OFFICERS (Biometrics)

£1,592 to £2,675

Applications are invited for a permanent and pensionable post in the Biometrics Division of the Department of Agriculture, Newforge Lane, Relfast

Applicants must be under 27 years of age on December 31, 1974 and have (1) a Degree, H.N.C. or equivalent qualification in Computing, Mathematics or Statistics; or (2) Stage I (formerly Part II) of the Examination leading to membership of the Institute of Statisticians.

The Biometrics Division supplies a consultant service to research workers of the Department on the statistical aspects of design and analysis of experiments and surveys. The post will be concerned with (a) the supervision of Assistant Scientific Officers who process research data collected by officers of the Department and post-graduate research students at the Agricultural and Food Science Centre, Newforge Lane, Belfast; and (b) the application of existing computer packages, the development of new computer programs and in the methodology of the direct capture of data for analytical procedures.

Starting salary will be related to qualifications and experience. A cost of living supplement is also payable.

There are prospects of promotion to Higher Scientific Officer (£2,461 to £3,371), Senior Scientific Officer (£3,157 to £4,441) and Principal Scientific Officer (£4,227 to £5,550).

Please write or telephone for an application form, quoting Ref. SB 314/74/N to Civil Service Commisssion, Clarendon House, Adelaide Street, Belfast BT2 8ND (telephone 0232-44300, ext. 26). Completed forms should be returned to arrive not later than December 5, 1974.





DEPARTMENT OF NUCLEAR MEDICINE

# PHYSICIST (Basic Grade)

required for Department engaged in a wide range of tests involving use of radioactive substances. The successful applicant will be initially concerned with commissioning a shadow-shield whole body counter, in collaboration with other physics staff, and carrying out measurements on patients.

Whitley Council conditions of service apply. Salary scale £1,689 to 22,562 p.a. plus London Weighting at £126 p.a. and current Threshold payments. Point of entry on scale will be determined by qualifications and experience.

Applications to Mr C. J. Hill, Personnel Department, Fulham Palace Road, Hammersmith, London, W6 8RF. Tel: 748 2050 ext. 2992. Please quote ref. 021. Forms should be returned by Décember 2, 1974.

#### MEDICAL RESEARCH COUNCIL CLINICAL AND POPULATION CYTOGENETICS UNIT SCIENTIST

A vacancy exists in the Experimental Studies Section of the above Unit for a research scientist, who will be principally concerned with the genetics/cytogenetics of human/murine hybrid cells, with emphasis on the mapping of human genes by somatic cell

The minimum qualification is an honours degree 2(1) in a biological science, although preference will be given to applicants with a Ph.D. or equivalent postgraduate experience. Previous experience of mammalian cell culture would be an advantage, but is not essential. Salary, dependent upon age, qualifications and experience will be on a scale £2,019 to £3,636 or £4,896 with F.S.S.U. benefits.

Applications in writing by December 16, 1974, giving full personal particulars and names of two referees, to the Administrative Officer, MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 (2XU.

The Australian Government Department of Minerals and Energy invites applications for the position of

# Director Bureau of Mineral Resources Geology and Geophysics

#### The Organisation

The Bureau, which forms part of the Department of Minerals and Energy, is the Australian Government's national earth sciences organisation. It carries out geological, geophysical and mineral resources surveys over most of Australia, its continental margin and its territories; undertakes experimental studies and research in geology and geophysics; and makes basic investigations of the earth's magnetic and gravitational fields, and in seismology and volcanology.

The Bureau currently has a staff of about 600, including 280 professional staff, mainly geologists and geophysicists. Headquarters are in Canberra, with appropriate research and laboratory facilities, and observatories and field stations are maintained in Darwin, Melbourne, Perth, Port Moresby and Antarctica. This year's budget is \$A4,500,000.

#### Duties

To direct the activities of the Bureau of Mineral Resources, which are to carry out geological, geophysical and geochemical surveys and research, and publish the results, to obtain basic information on and review the geology of Australia and its mineral resources.

#### Qualifications

Wide experience in carrying out and directing investigations in the earth sciences and in mineral resources. Administrative ability of a high order.

Appropriate academic qualifications.

Salary \$21,417 (Australian) (at present exchange rate £1 = \$A1.79 approx.)

#### Location Canberra.

Conditions of Service include Four weeks annual leave plus bonus, liberal sick leave, three months furlough after ten years service, removal expenses to Canberra.

#### Appointment

Permanent appointment to the Australian Public Service is available to British subjects eligible for permanent residence in Australia who will contribute to a comprehensive superannuation scheme after appointment.

Temporary engagements for fixed periods may be considered.

Enquiries, or applications giving full details of qualifications and experience and names of referees, should be forwarded to: The Secretary, Department of Minerals and Energy, P.O. Box 5, CANBERRA A.C.T. 2600 Australia. Closing date 13th December 1974.

#### **PHARMACOLOGIST**

experienced, with veterinary or medical qualifications preferred, required for pharmacological research and toxicological studies of compounds of potential therapeutic importance. Excellent opportunities advancement in modern, well-equipped laboratory, also vacancies for New Graduates as trainees. Pension and Assurance Scheme. Application forms from: The Secretary, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London N1 2HB.

(1997)

#### UNIVERSITY OF NAIROBI-KENYA

UNIVERSITY OF NAIROBI—KENYA

Applications are invited for posts of (a) 2
SENIOR LECTURERS and (b) 3 LECTURERS
IN DEPARTMENT OF PHARMACY. Applicants
for (a) should have a degree in Pharmacy and a
higher degree in either Pharmacology and Toxicology or in Pharmacy. Preference will be given
to those with previous teaching at university
level and who have much research experience in
their fields. Applicants for (b) should have a
degree in Pharmacy and a postgraduate degree in
any of the following:—Pharmacy and Toxicology;
Pharmacognosy; Pharmaceutics; Pharmaceutical
Chemistry; Pharmacy. Pharmacists who are interested in these jobs will be considered for
Assistant Lectureship. Appointees will be expected
to teach both undergraduates and postgraduates
and carry out research in their fields. Salary
scales: Senior Lecturer K£2,256 to K£3,036 p.a.
Lecturer K£1,260 to K£2,580 p.a. Assistant
Lecturer K£1,260 to K£1,440 p.a. (K£1=£1.19
sterling). The British Government may supplement
salaries of Senior Lecturers and Lecturers in range
£900 to £1,752 p.a. (sterling) for married appoint
ees of £204 to £948 p.a. (sterling) for single
appointees (normally free of all tax) and provide
children's education allowances and holiday visit
passages. This supplementation is unlikely to be
applied to appointments at Assistant Lecturer
level. F.S.S.U. Family passages; various allowances. Detailed applications (2 copies) including
a curriculum vitae and naming 3 referees, should
be sent by airmail, not later than December 23,
1974 to Registrar, University of Nairobi, P.O. Box
30197, Nairobi, Kenya. Applicants resident in
U.K. should also send 1 copy to Inter-University
Council, 90/91 Tottenham Court Road, London
WIP ODT. Further particulars may be obtained
from either address. (1999)

#### UNIVERSITY OF PAPUA **NEW GUINEA**

NEW GUINEA

Applications are invited for LECTURESHIP IN PHYSICAL GEOGRAPHY to teach courses at the introductory and advanced levels. Preference will be given to candidates with interests in Geomorphology, Climatology or applied aspects of the subject. The ability to help in courses in geographical techniques and some experience in aerial photograph interpretation, are desirable. Ample field opportunities exist, and the Department has its own 4-wheel drive vehicle, boat and the normal complement of equipment. Salary scale: (including overseas allowances) A\$9,174 to A\$11,862 p.a. plus A\$360 p.a. dependants allowance (£1 sterling=A\$1.78). Conditions include provision of housing, study leave, annual leave fares, F.S.S.U. Applications (2 copies) should include particulars of age, nationality, marital status, academic record, qualifications and experience, names and addresses of three referees, a recent small photograph and an indication of estimated starting date. Further details and conditions of appointment are available from K. R. Long, Secretary, P.O. Box 4820, University, Papua, New Guinea. Closing date December 20, 1974. (1998)

#### UNIVERSITY OF DURHAM DEPARTMENT OF MATHEMATICS

Applications are invited for the post of POST-DOCTORAL SENIOR RESEARCH ASSISTANT to work on numerical methods for solving a class of integrodifferential equations arising in atomic scattering theory. The post is tenable for two years from April 1, 1975, Applicants should have suitable research experience in numerical analysis.

Starting salary £2,055 plus threshold payments and F.S.S.U. benefits.

Applications (3 copies) naming three referees, should be sent by December 12, 1974 to the Registrar and Secretary. Science Laboratories, South Road, Durham, DHI 3LE, from whom further particulars may be obtained. (2004)

#### CHAIR OF ANATOMY

#### UNIVERSITY OF NORTH CAROLINA

Applications and nominations are invited for the position of Professor and Head of the Department of Anatomy in the School of Medicine. Candidates must have a generally recognised record of important scientific contributions and a continuing commitment to vigorous investigative activity. It is expected that a candidate will have a broad background in anatomy and cell biology. The department has responsibility for teaching of gross and microscopic anatomy, neurobiology, reproductive biology and embryology in the Schools of Medicine and Dentistry and to Allied Health Disciplines. The successful candidate therefore must be seriously interested in medical and paramedical education and be prepared to assume active leadership in developing and implementing innovative and effective teaching programs for these areas and for graduate students.

Interested persons may obtain further informa-

these areas and for graduate students.

Interested persons may obtain further information or submit letters of application or nominations accompanied by a detailed curriculum vitae to CHAIRMAN, ANATOMY SEARCH COMMITTEE, DEAN'S OFFICE, UNIVERSITY OF NORTH CAROLINA SCHOOL OF MEDICINE, CHAPEL HILL, NORTH CAROLINA 27514. An equal opportunity, affirmative action employer. (2006)

The BOTSWANA AGRICULTURAL COLLEGE, situated 7 miles from Gaborone, invites applications for a

#### SENIOR INSTRUCTOR

to head the Crops Section and manage both the theoretical and practical teaching of crop husbandry, horticulture and veld management as part of the 2-year Agriculture Certificate Course which approximates to the U.K. National Certificate in Agriculture.

Candidates should have a Diploma in Agriculture or allied subject. If possible they should have teaching experience and have had relevant experience in a developing country.

The post is tenable for 2-3 years commencing as soon as possible.

Salary in range £2,350 to £4,502 p.a. inclusive of a normal tax-free supplement paid by the British Government to citizens of, and permanently resident in, the U.K.

Benefits include a 25% terminal gratuity on the basic salary; free passage; education allowances; holiday visit passages; subsidised accommodation; generous leave and in certain circumstances, an appointment grant and interest free car loan.

Apply for further details to the Appointments Officer, T.E.T.O.C., (The Council for Technical Education and Training for Overseas Countries), 35/37 Grosvenor Gardens, London, SWIW 0BS.

(2005)

# THE POLYTECHNIC OF CENTRAL LONDON

SCHOOL OF ENGINEERING AND SCIENCE

#### RESEARCH ASSISTANT

£1,544 to £1,654 plus Threshold Agreement

for the Physics Unit. The successful candidate will investigate the solubility of deuterium in vanadium using ultra-high vacuum techniques and use X-Ray crystallographic methods to determine the strain produced in the metal lattice by the dissolved deuterium. He/she will be expected to register for the degree of Ph.D. Applications are invited from honour graduates.

Details and application form from The Establishment Officer, PCL, 309 Regent Street, London W1R 8AL. Tel: 01-580 2020 Ext. 212. (2017)

# Opportunity Overseas

# Sierra Leone

### **Production Manager (Forestry)**

At the Forest Industries Corporation, to carry out stock surveys of exploitable timber trees; plan and execute log extraction programmes; organise conversion and seasoning in the sawmill and where necessary assist in marketing timber produce; organise the operation of the furniture factory and provide advice and guidance to a counterpart.

Applicants should have experience in the prescribed duties of the post with some experience of furniture manufacture or similar type of wood processing. Appointment three years. Salary of not less than £6,000 p.a. plus a variable tax-free allowance in scale £1,005 to £2,500 p.a.

Other benefits include paid leave, free family passages, children's education allowances and free accommodation and medical attention. Applicants should normally be citizens of and permanently resident in the United Kingdom.

For full details and an application form please apply giving age and brief details of qualifications and experience to:-

Appointments Officer,
Ministry of

Overseas Development

Room E301, Eland House, Stag Place, London SW1E 5DH (1993)

# Find your place in British Gas

# SCIENTIST/ENGINEER

The Physics Division at London Research Station has a vacancy for a Scientist/Engineer to work on the measurement and prediction of thermodynamic properties of natural gas mixtures. These measurements are necessary for the efficient design and operation of systems for the liquefaction, storage, transmission and distribution of natural gas at high temperatures.

Candidates should have a good honours degree in Science or Engineering; starting salary will be according to qualifications and experience, but will be at a point on scales which rise to £4,197, plus threshold supplement.

For further details and an application form, write to the Research Secretary, British Gas, London Research Station, Michael Road, Fulham, London SW6 2AD, quoting 4010/15/NA.

(2024)

**BRITISH GAS** 

#### ROYAL ASTRONOMICAL SOCIETY LEVERHULME VISITING FELLOWSHIPS IN ASTRONOMY 1975–1976

The Council of the Royal Astronomical Society is prepared to receive applications for Leverhulme Visiting Fellowships in the academic year 1975-76 (or the calendar year 1976). These awards are open to scholars from the Commonwealth and foreign countries, with a preference for the latter and for those who have not previously had the opportunity of an extended stay in the U.K., who wish to undertake a year's study in any branch of astronomy at a university or another approved institution in the United Kinedom.

Not more than two such Fellowships will be available. The stipend attaching to an award will be in the range £2,600 to £3,000. A contribution towards the cost of the successful applicant's travelling expenses will be made.

Application forms, which must be completed and returned by January 31, 1975, may be obtained from: The General Secretary, Royal Astronomical Society, Burlington House, London WIV 0NL. It is hoped to inform successful candidates by early April 1975.

# UNIVERSITY OF BRISTOL DEPARTMENT OF PATHOLOGY

# POSTDOCTORAL RESEARCH ASSOCIATE

A postdoctoral research associate is required to work in the Comparative Pathology Laboratory, School of Veterinary Science, Langford, Bristol, in collaboration with Dr D. J. Etherington of the adjoining Meat Research Institute (A.R.C.) on the purification, biochemical properties and mode oction of a lysosomal collangenolytic cathespin. This is a funded post, supported by a grant, initially for three years, from the Arthritis and Rheumatism Council, with a salary on the scale £2,580 to £2,931 per annum.

Applications in writing giving details of age,

Applications in writing, giving details of age, qualifications and research experience, plus the names of two referees, to Professor I. A. Silver, Pathology Department, Medical School, University Walk, Bristol BS8 1TD. (2014)

# Anglo-Australian Telescope Board

The Anglo-Australian Telescope Board invites applications for vacant positions at its scientific headquarters operations, currently located in Epping, near Sydney. In addition to its Epping site, the Board operates a 3.9 metre optical Telescope in the Warrumbungle mountains near Coonabarabran, about 200 miles north-west of Sydney. The Telescope is maintained by a resident site organisation but from time to time it will be necessary

# Assistant Librarian or Librarian Class 1

Duties will include cataloguing scientific and technical publications; maintaining the periodical subscriptions; ordering books, etc. Applicants should have a University degree (preferably in science) or attained Associateship of the Library Association or passed one of the professional examinations of the Library Association.

Salary Range \$A 5290 to 9262

# **Assistant Photographer**

Duties will include testing of astronomical plates; assisting the astronomical staff; preparing prints and slides from astronomical negatives and overseeing the purchasing and preparation of photographic chemicals and materials. Applicants should have diversified photographic experience, although experience in scientific laboratories would be valuable.

Salary Range \$A 7756 to 11360

#### **Tool Room Machinist**

The successful applicant will assist the scientific and technical staff in maintaining and instrumenting the new Anglo-Australian Telescope. Experience in working with a scientific group in developing new equipment will be valuable. Applicants should have had extensive experience on all types of machine tools. Salary Range \$A 6790 to 8696

### **Electronics Technician**

The successful applicant will assist the scientific and technical staff in maintaining and instrumenting the new Anglo-Australian Telescope. Applicants should have an ONC in Electrical Engineering, or an equivalent qualification, and have served an apprenticeship followed by at least 3 years' experience in a variety of electronic fields, but with an emphasis on digital electronics.

Salary Range \$A 7756 to 10495

for headquarters personnel to travel to site for a few days to assist the site organisation. The tenure of these appointments is for an indefinite period and carries superannuation benefits. Appointees recruited to permanent positions from outside Sydney will have their fares to Sydney (and those of their dependants) paid by the AAT Board. The vacant positions include:—

### **Design Draughtsman**

The successful applicant will work with the AAT scientific and engineering staff in the preparation of designs and drawings for the AAT. Applicants should have an ONC or an equivalent qualification and have served an apprenticeship or had equivalent training for at least 3 years, together with at least one year's drafting experience. Experience in working with other scientific organisations would be valuable.

Salary Range \$A 7756 to 10495

### Mechanical/Optical Engineer

The successful applicant will be expected to assist the scientific staff of the AAT in the design and construction of new instruments for the AAT. Many of these instrument will be novel, experimental devices and will require a high degree of engineering ingenuity for their successful development. In addition the successful applicant will be expected to support the staff in maintaining the effectiveness of the Telescope.

Applicants should posses a degree in engineering or have passed the Council of Engineering Institutions Part 2 examination in appropriate subjects or should possess an equivalent academic qualification, together with extensive engineering design and drawing office experience.

Salary Range \$A 10465 to 14049

### **Electronics Engineer**

The successful applicant will be expected to assist the scientific staff of the AAT in the design and construction of new instruments for the AAT. Many of these instruments will be novel, experimental devices and will require a high degree of engineering ingenuity for their successful development. In addition, the engineering staff is expected to support the site staff in maintaining the effectiveness of the Telescope.

Applicants should possess a degree in electronic engineering or physics or have passed the Council of Engineering Institutions Part 2 examination in appropriate subjects or should possess an equivalent academic qualification, together with several years' experience in circuit design, digital logic and computer operations.

Salary Range \$A 7000 to 14049

Applications, giving details of qualifications and experience and the names and addresses of 3 referees, should be made by letter to:---

The Executive Officer, Anglo-Australian Telescope Board c/o Department of Science, PO Box 449, Woden, ACT 2606, Australia by 13 December, 1974

#### FIELD STUDIES COUNCIL DEPUTY WARDEN

at Juniper Hall Field Centre, near Dorking. Good degree in biological science, educational qualifications. At least two years professional teaching experience including fieldwork. Wide environmental interests, but special knowledge of botany an asset.

interests, but special knowledge of botany an asset. Besides teaching, duties include administration (staff, student supervision, programme planning, equipment, etc.) in consultation with Warden as his second in command. Research opportunities. Salary £1,800 by £75 to £2,025 plus free board and lodging. Appointment January 1975, closing date for applications November 30, 1974. Further details and application forms from the Director, Field Studies Council, Preston Montford, Montford Bridge, Shrewsbury SY4 1HW. (2013)

#### YALE UNIVERSITY REPRODUCTIVE BIOLOGY

Department of Obstetrics and Gynaecology Department of Obstetrics and Gynaecology seeks a senior experienced investigator to lead reorganisation and direction of departmental programme in reproductive biology. New laboratory and animal facilities available. Strong clinical endocrinology, receptor pharmacology, electron microscopy and in witro fertilisation elements already functioning. Appointment at Associate Professorship level (university funded) with joint appointment of appropriate individual in basic science departments with access to graduate students, and participation in university-wide reproductive biology centre. In accord with Yale University's Affirmative Action Programme, the search committee welcomes women and minority group candidates.

Applicants should submit curriculum vitae and

Applicants should submit curriculum vitae and bibliography to Nathan Kase, M.D., Chairman of Search Committee, Department of Obstetries and Gynaecology, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510.

#### UNIVERSITY OF THE WITWATERSRAND Johannesburg, South Africa

#### LECTURER IN MICROBIOLOGY

Applications are invited for the above post in the Department of Botany and Microbiology. A special knowledge in microbial industrial applications or microbial metabolism would be an advantage, but not essential for the position offered

The two-year degree course, which is in the process of development, caters for both pure and applied aspects of microbiology.

The salary scale attached to the post is R6,300 by 360 to R 9,180 (£1=R1.62), and the initial salary will be determined according to qualifications and experience. Benefits include an annual bonus, pension and medical aid facilities and a housing subsidy (if eligible).

housing subsidy (if eligible).

Intending applicants should obtain the information sheet relating to this post. The policy of the University is not to discriminate in the appointment of staff or the selection of students on the ground of sex, religion, race, colour or national origin. Further particulars relating to this policy and the extent to which it can be implemented in practice, are included in the information sheet, which should be obtained from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa, with whom applications should be lodged not later than January 10, 1975, U.K. applicants may obtain the information sheet relating to this post from the London Representative, University of the Witwatersrand, 278 High Holborn, London WCI. (2008)

#### MEDICAL RESEARCH COUNCIL

#### SCIENCE GRADUATE

#### FOR BIOCHEMISTRY DIVISION

The National Institute for Medical Research has a vacancy for the post of JUNIOR TECHNICAL OFFICER in the Biochemistry Division. The research being carried out is concerned with the preparation and characterisation of mammalian cell membranes. As a theoretical understanding of the research would be useful, applicants should preferably have graduated in biochemistry or have studied a biological subsidiary. Salary scale from £1,875 p.a. plus £167 p.a. threshold agreement (maximum £2,718 plus £167 p.a.). To obtain application forms please write quoting ref: JTO/BM to Mrs M. Y. Jim, Assistant Personnel Officer, N.I.M.R., The Ridgeway, Mill Hill NW7 1AA. Tel: 01-959 3666. (2018)

#### **GRIFFITH UNIVERSITY** Brisbane, Australia

#### LECTURESHIP-EXPERIMENTAL PHYSICS SCHOOL OF SCIENCE

Griffith University will admit its first undergraduates in March 1975 and is committed to interdisciplinary teaching methods. The appointee, in addition to first year teaching duties, will have a particular responsibility, in collaboration with existing staff, for the development of second year courses. The research interests of the School are in solid state physics, chemistry and biochemistry. The appointee should be prepared to be associated with interdisciplinary research projects, in addition to pursuing his own research interests. Applications are invited from men and women for this position, which closes on **December 16**, 1974.

The appointment will be from March 1, 1975, or as soon thereafter as possible, at a salary in the range \$A9,002 to \$12,352 (currently under review). This appointment will be made in the lower half of the range.

Further information, conditions of appointment and details of the method of application can be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF. (2010)

#### JAMES COOK UNIVERSITY OF NORTH QUEENSLAND TECHNICAL OFFICER SCHOOL OF BIOLOGICAL SCIENCES

The successful applicant will be responsible for the general management and maintenance of the equipment and supplies of the Departments of Botany, Marine Biology and Zoology as well as for the training and supervision of all the technical staff of the Sobool. Applicants should be over the age of 25 years, have a technological diploma or equivalent and at least five years' experience in a biological laboratory, preferably with some of this time in an administrative capacity. An interest in Marine Biology research would be an advantage.

Marine Biology research would be an advantage.

Sallary ranges are: Technical Officer Grade 1—
\$A319.10 to \$344.40 per fortnight (\$A8,323 to \$8,983 p.a.); Technical Officer Grade 11—\$A353.20 to \$379.60 per fortnight (\$A9,213 to \$9,903 p.a.).

A locality allowance of \$A142 p.a. for a married male or \$A71 p.a. for a single appointee also payable. Conditions of appointment include F.S.S.U. superannuation, invalid pension scheme, housing assistance, study leave and allowance for travel and removal expenses on appointment.

Further details and application forms obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WCIH 0PF.

Applications close December 6, 1974. (2011)

#### Department of Health and Social Security, London

■ In Hospital Scientific and Technical Services Unit ■ Assist in the formulation of policies in the laboratory science field of microbiology, haemotology and clinical chemistry.

☐ 1st/2nd hons degree or equivalent in appropriate scientific subject ☐ 4 years' post-graduate experience, including some in a hospital laboratory  $\square$  Age under 32  $\square$  Appointment as Senior Scientific Officer (around £3550 to over £4800)  $\square$  Ref: SB/3/K.

Application forms (for return by 13 December 1974), from Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, telephone Basingstoke 29222 ext. 500 (or, for 24 hour answering service, London 01-839 1992).

#### Department of the Environment Central Water Planning Unit, Reading

# Chemists

■ In Water Quality Division ■ Appraise capacities, tolerances and failure rates of water supply treatment and effluent treatment methods Assess suitability of water sources for potable and industrial supplies ■ Relate water quality data to methods of effluent treatment, land use practices and reservoir operations ■ Evaluate implications of alternative strategies in the context of river water quality, water treatment and effluent treatment. 

Possibly of collaboration with water systems analysis on the development of water quality models.

☐ 1st/2nd hons degree or equivalent in Chemistry or corporate membership of an appropriate institution  $\square$  Experience of either water supply chemistry, effluent water chemistry or river water chemistry desirable  $\square$  Age under 32  $\square$ Appointment as Senior Scientific Officer (over £3300 to around £4600) or Higher Scientific Officer (£2600 to over £3500) according to age and experience Ref: SA/7/HF.

☐ Application forms (for return by 13 December 1974), from Department of the Environment, Room 446, Lambeth Bridge House, Albert Embankment, London SE1. Telephone 01-735 7611 ext. 1469 or 2094.



#### UNIVERSITY OF WESTERN AUSTRALIA Perth

PHYSIOLOGY

Applications are invited for appointment as LECTURER or SENIOR LECTURER

LECTURER or SENIOR LECTURER
in the Department of Physiology. Applicants
interested in joining the auditory physiology and
biophysics group would be particularly welcome.
The laboratory is exceptionally well equipped for
investigating all functions of the peripheral
auditory system and could also provide facilities
for study of the central auditory pathway. The
current salary ranges are: Lecturer—\$A9,002 to
\$A12,352 p.a.; Senior Lecturer—\$A9,002 to
\$A14,724 p.a. Benefits include superannuation
similar to F.S.S.U. fares to Perth for appointee
and dependent family, removal allowance, study
leave and long service leave and housing loan
scheme. Further information may be obtained
from the Staffing Officer.

Applications in duplicate stating full personal
particulars, qualifications and experience should
reach the Staffing Officer, University of Western
Australia, Nedlands, Western Australia 6009, by
January 11, 1975. Candidates should request three
referees to write immediately to the Staffing
Officer. (2009)

#### UNIVERSITY OF THE WITWATERSRAND Johannesburg, South Africa

CHAIR OF BOTANY

CHAIR OF BOTANY

Applications are invited for an additional Chair of Botany. Preference will be given to persons who have research interests in one of the modern aspects of the plant sciences.

The salary attached to the post is in the range R10,800 by 450 to R12,600 by 600 to R13,800 (£1=R1.62), the initial salary to be determined according to qualifications and experience.

Intending applicants should obtain the information sheet relating to this post. The policy of the University is not to discriminate in the appointment of staff or the selection of students on the ground of sex, religion, race, colour or national origin. Further particulars relating to this policy and the extent to which it can be implemented in practice, are included in the information sheet, which should be obtained from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa, with whom applications should be lodged not later than January 6, 1975. U.K. applicants may obtain the information sheet relating to this post from the London Representative, University of the Witwatersrand, 278 Tigh Holborn, London WC1.

FIELD STUDIES COUNCIL TUTOR

TUTOR
in Geography or Geology at Juniper Hall Field Centre, Dorking. Single, good degree, emphasis on physical aspects. Educational qualifications desirable. Research opportunities. Salary £1,200 by £75 of £1,330 plus free board and lodging. Appointment January 1975. Closing date for applications November 30, 1974. Further details and application forms from the Director, Field Studies Council. Preston Montford, Montford Bridge, Shrewsbury SY4 1HW. (2012) (2012)

#### UNIVERSITY OF LEEDS DEPARTMENT OF EXPERIMENTAL PATHOLOGY AND CANCER RESEARCH **TECHNICIAN**

required to assist a group engaged in developing short-term tests for chemical carcinogens. Candidates should have a background in microbiology, biochemistry, or mammalian cell culture. The position, which is funded by The M.R.C., is for 3 years and the salary will start at the appropriate point on the grade 3 scale (£1,650 to £1,920, plus threshold payments).

Applications in writing giving details of career should be sent to Dr R. C. Garner, University of Leeds, Leeds LS2 9JT. (2016)



# **Biologist**

### for Environmental work about £4500

BP Chemicals International require a biologist to join their environmental group based in Central London.

This group is responsible for providing advice to management on all environmental matters and ensuring that all necessary information on this subject is available to assist decision making. Important parts of this work are monitoring the effect of company activities in areas close to plants and considering biological methods of treating certain wastes. It is a small group in which members need to be able to operate independently in discussions at senior level both within the company and with outside bodies.

Candidates must have a degree in a biological science followed by a number of years' industrial experience, which has included work of a biological nature. The successful applicant will need to become acquainted with the biological aspects of air and water quality standards and with the major processes operated by the company.

Starting salary will be around £4500 including Inner London Allowance. In addition there is a threshold payment of £166. Career development prospects within the BP group of companies are excellent and the conditions of employment include a non-contributory pension, 4 weeks' holiday and an excellent subsidised restaurant.

Please write or telephone for an application form to: E. P..A. Norman, BP Chemicals International Ltd., Devonshire House, Piccadilly, London W1X 6AY. Tel: 01-629 8867, ext. 76.

**BPchemicals** 



#### THE DEPARTMENT OF PSYCHOLOGY PRINCETON UNIVERSITY

anticipates several openings for the 1975–76 academic year. One is at the Full Professor level in social psychology, where we seek an addition to our ongoing programme in social psychology. The other(s) at the Assistant Professor level are in ne other(s) at the Assistant Professor level are in general experimental psychology, with concentration in animal behaviour, developmental and comparative psychology, or personality research. These latter anticipated openings are not rigidly tied to any specific area of expertise. Sheer excellence in research and teaching will override area-of-interest considerations.

Nominations and applications should be sent to the Chairman, Department of Psychology, Green Hall, Princeton University, Princeton, New Jersey 08540. We are an equal opportunity employer with a vigorous affirmative action programme. (2022)

#### UNIVERSITY OF NAIROBI Kenya

Applications are invited for the following posts in the DEPARTMENT OF ANIMAL PRODUCTION:—

DUCTION:—

1. ASSOCIATE PROFESSOR. Applicants, who must have a Ph.D. in either Animal Nutrition, Genetics and Breeding, or Ecology and Management, should also possess a good general background in the other branches of Animal Production. They must have done several years of relevant university teaching and have experience in directing postgraduate students. A knowledge of production systems in the tropics, preferably in East Africa will be an advantage but not essential.

of production systems in the tropics, preferably in East Africa will be an advantage but not essential.

2. THREE SENIOR LECTURERS. Applicants should have a good first degree in either Veterinary Medicine or Agriculture and preferably a Ph.D. Candidates holding lower qualifications (not less than an M.Sc.) will only be considered if they have several years research and teaching experience at university level, in either Animal Feeding, Management or Breeding.

3. LECTURER IN ANIMAL BREEDING. Applicants should have a good first degree in either Veterinary Medicine or Agriculture and at least an M.Sc. in the field of Animal Breeding. Experience in teaching at university level will not be necessary for holders of a Ph.D. in this field. Salary scales: Associate Professor K£3,036 to K£3,564 p.s. Senior Lecturer K£2,256 to K£3,036 p.a. Lecturer K£1,500 to K£2,580 p.a. (K£=£1.19 sterling). The British Government may supplement salaries in range £900 to £1,860 p.a. (sterling) for single appointees (normally free of all tax), and provide children's education allowances and holiday visit passages, FS.S.U. Family passages; various allowances. Detailed applications (2 copies) including a curriculum vitae and naming 3 referes, should be sent by airmail, not later than December 30, 1974, to the Registrar, University of Nairobi, PO Box 30197, Nairobi, Kenya. Applicants resident in UK should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London WIP ODT. Further particulars may be obtained from either address. (2026)

#### FELLOWSHIPS AND **STUDENTSHIPS**

#### RHEUMATOLOGY CLINICAL TRAINING FELLOWSHIP

Division of Rheumatology of the Department of Medicine, S.U.N.Y. is accepting applications for January 1, 1975 appointment. Inter-disciplinary rheumatic study involving the departments of Pathology, Medicine and Bio-Medical Engineering has been established offering major opportunities in research and clinical activities. E.C.F.M.G. required. Starting salary \$18,000 per year. Applications should be sent to Leonard E. Meiselas, M.D., Professor of Medicine, Project Director, School of Medicine, Health Sciences Center, State University of New York at Stony Brook, Stony Brook New York 11794, An Equal Opportunity/Affirmative Action Employer. (1956)

#### UNIVERSITY OF LEICESTER DEPARTMENT OF PHYSICS

DEPARTMENT OF PHYSICS

An S.R.C./C.A.S.E. studentship is available for studies in the fields of ionospheric physics and radiowave propagation leading to a higher degree. The project is related to current research in the field of high frequency communications and is sponsored by a Government Laboratory.

Applications are invited from graduates in physics, mathematics or electrical engineering and should be sent to Dr. T. B. Jones, Department of Physics, University of Leicester, Leicester LEI 7RH. (1964)

#### THE ROYAL SOCIETY

John Murray Travelling Studentship in Oceanography and Limnology 1975

Applications are invited for the above Student-Applications are invited for the above Studentship, open to male or female graduates under the age of 35, for the encouragement of travel and work in oceanography or limnology. This award, which may be held for periods from three months to one year, will in 1975 be up to £1,000, adjusted according to qualifications and place and duration of research. Applications are especially invited from workers in oceanography and immology, as well as in zoology, botany, geology, mathematics and physics, and it will be permitted to hold the award while receiving a salary from a regular appointment. regular appointment.

Applications receivable by December 15, 1974, should be made on forms obtainable from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG. (1952)

#### UNIVERSITY OF ABERDEEN RESEARCH FELLOW IN ENGINEERING SCIENCE

Applications are invited for the above post, to work with Professor J. F. Eastham on linear induction motors.

The post is financed by the S.R.C. and is tenable for three years. Salary within the range £2,118 to £2,412 plus threshold payments.

Further particulars from The Secretary The University, Aberdeen, with whom applications (3 copies) should be lodged by December 14, 1974.

(1981)

#### POSTDOCTORAL RESEARCH **FELLOWSHIP**

is available for working on the chemical basis of single protein-RNA interactions in bacterial ribosomes. Previous research on nucleic acid modification or sequence determination, or in the fields of physical chemical or X-ray crystallographic studies on proteins or RNA is preferred.

The Fellowship commences on about April 1, 1975 and continues for about two years. Knowledge of German is not essential. The salary is approximately £5,000 per year and is taxable.

Applications should be made in writing, with details of previous research experience and current interests, to Dr R. A. Garrett, Max-Planck-Institut für Molekulare Genetik, Ihnestrasse 63/73, 1 Berlin 33 (West). (1974)

#### UNIVERSITY OF READING PLANT SCIENCE LABORATORIES DEPARTMENT OF BOTANY

#### S.R.C./C.A.S.E. STUDENTSHIP IN **PHYTOCHEMISTRY**

Applications are invited from Honours Graduates in Organic Chemistry, Biochemistry or Botany to work on "Comparative Aspects of Phytoalexin production in the family Leguminosae" in association with I.C.I. Plant Protection Ltd., Jealott's Hill Research Station, Bracknell, Berks.

The studentship is available immediately for a period of up to three years.

Application with a curriculum vitae and the names of two referees should be sent to:

Dr J. B. Harborne, Phytochemical Unit, Plant Science Labs., University of Reading, Whiteknights, Reading RG6 2AS

(1990)

#### UNIVERSITY OF DUNDEE

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

#### POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited from candidates with experience of biochemical techniques for a postdoctoral research fellowship in the above department based at Ninewells Hospital and Medical School, Dundee. The appointment which will be for 3 years will involve work in a research project on the effect of drugs on red blood cells and the protective effects of the enzyme systems. The initial salary will be £2,118 per annum.

annum.
Further information can be obtained from Dr. G. R. Tudhope at the Department of Pharmacology and Therapeutics, Ninewells Hospital Dundee, DD2 1UD.
Applications, together with a curriculum vitae and the names of two referees, should be sent to The Secretary, The University. Dundee, DD1 4HN, by December 13, 1974.

(1968)

#### UNIVERSITY OF EDINBURGH RESEARCH ASSISTANT

required by the DEPARTMENT OF MOLECULAR BIOLOGY to join a research team studying protein synthesis in relation to cell division in bacteria. Applicants should possess cither H.N.C., A.I.M.L.T. or B.Sc. (Hon) in appropriate subjects.

The post is grant supported for three years in the first instance with a possibility of renewal thereafter. Salary will be on scale £2,247 to £2,628 or £2,439 to £2,895.

Applications, quoting the post reference number (A085), should be addressed to the Personnel Officer, University of Edinburgh, 63 South Bridge, Edinburgh EH1 ILS. Tel 031-667 1011 extn. 4446. (2015)

#### POSTDOCTORAL FELLOWSHIPS DEPARTMENT OF PHYSICS

UNIVERSITY OF WATERLOO Waterloo, Ontario, Canada

Applications are invited for postdoctoral research fellowships in physics.

Fellowships carry a stipend of \$9,600 per annum (after March 31, 1975) which is subject to Canadian income tax. The awards are tenable for a period of one year with a possible renewal for a second

Research areas include: Astronomy and Astrophysics (applicants in this area should be interested in teaching and in observational galactic structure of Southern Milky Way and related topics), Atomic and Molecular Physics, Biophysics, Crystallography, Electron Microscopy (thin crystal defects and interfacial structures), Ellipsometry, Condensed Inert Gas Properties, Laser Physics, Microwave Physics, Nuclear Magnetic Resonance, Solid State Physics (insulators, motals, non-linear dielectrics, and semiconductors), Statistical Mechanics, Superfluidity, Superconductivity, and Ultrasonics. Closing date for applications is March 15, 1975.

For further information write to:

Dr D. E. Brodie
Acting Chairman
Department of Physics
University of Waterloo
Waterloo, Ontario, Canada N2L 3G1
(2021)

#### ALCOHOLISM M.C.A. RESEARCH FELLOWSHIP

The Council offers a Fellowship of 2 or 3 years to a medically or scientifically qualified worker for research into the cause, detection or prevention of alcohol dependence. The financial support will be adjusted to the experience of the worker and the demands of the proposed research. Full information from: The Executive Director, The Medical Council on Alcoholism, 8 Bourdon Street, London WIX 9HY. (1864)

#### LECTURES AND COURSES

#### UNIVERSITY OF LONDON

A course of three lectures entitled 1. "Vortex Dynamics" 2. "Setting of Dilute Suspensions in Viscous Fluids" 3. "Hydrodynamics of Thin Films of He 11" will be delivered by Professor P. G. Saffman (California) at 5.30 p.m. on December 2, 4, 5, at Imperial College & Science and Technology (Mechanical Engineering Department), Prince Consort Road, SW7.

ADMISSION FREE, WITHOUT TICKET Academic Registrar (1982)

#### UNIVERSITY OF LONDON

A course of two lectures entitled 1. "Geography of Disease in Developing Lands" 2. "Geography of Disease in Developed Countries" will be delivered by Professor G. Melvyn Howe (Strathclyde) at 5.15 p.m. on November 27 and 28 at Queen Mary College, (Chemistry Lecture Theatre), Mile End Road, El.

ADMISSION FREE, WITHOUT TICKET

Academic Registrar

(1983)

#### UNIVERSITY OF LONDON

A lecture entitled "The Geochemistry of Basaltic Rocks" will be delivered by Dr S. R. Hart (Washington) at 5.30 p.m. on Tuesday, December 3 at Imperial College of Science and Technology, (Large Lecture Theatre), Prince Consort Road,

ADMISSION FREE, WITHOUT TICKET
Academic Registrar

(1984)

#### UNIVERSITY OF LONDON

A lecture entitled "Deep Drilling into Oceanic Islands: Bermuda and the Azores" will be delivered by Dr. F. Aumento (Canada), at 5.30 p.m. on Tuesday December 3 at Imperial College of Soience and Technology (Geology Department), South Kensington, S.W.7.

ADMISSION FREE, WITHOUT TICKET

Academic Registrar (2000)

#### **MISCELLANEOUS**

#### SABBATICAL?

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#### **GRANTS & SCHOLARSHIPS**

#### BROWNE RESEARCH FUND MAURICE HILL RESEARCH FUND Grants 1975

The Council of the Royal Society invites applications from qualified research workers for personal grants

- cations from qualified research workers for personal grants

  (a) from the Browne Research Fund, for research in marine biology at a marine biological laboratory or elsewhere. Preference will be given to those who propose to work on purely scientific problems rather than to those whose work would be primarily directed towards an economic end.

  (b) from the Maurice Hill Research Fund (established in memory of the late Dr. M. Hill, F.R.S., for the encouragement of research in physical oceanography and marine geophysics) for research at sea and/or in the laboratory in physical and chemical oceanography, marine geophysics and geology.

  Grants may be used for periods up to one year, and are made on the understanding that university or other posts held by applicants will meanwhile be kept open for them.

  In 1975 the amounts available will be about £4,000 from the Browne Research Fund and £2,500 from the Maurice Hill Research Fund.

  Applications should be made on forms to be obtained from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, and returned by December 15, 1974. (1951)

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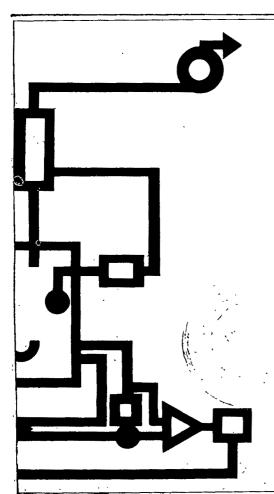
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### **Gas Analysis Instrumentation**

Anthony Verdin

The detection and analysis of gases and vapours has become essential in many areas of modern life; noticeably industrial safety and control of the environment. As a result a whole class of physio-chemical methods has come into existence. The author draws on his fourteen years of experience in the field to supply the only wide-ranging view of all types of equipment presently available. Both academics and industrialists will find it indispensable.

#### Electrical Breakdown in Gases

Edited by J. A. Rees

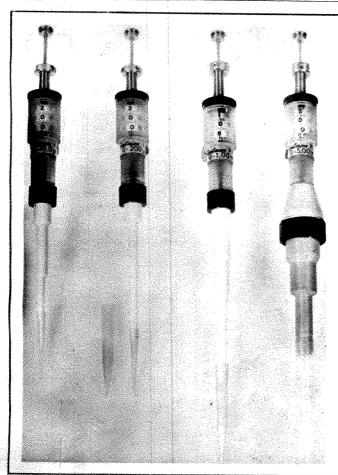
The electrical breakdown of gases has been studied in the laboratory since before the start of this century and it is sixty years since Sir J. S. Townsend wrote his monograph The Theory and Ionisation of Cases by Collision. In that time the subject has been widely studied and a great deal of progress has been made in understanding many aspects of the problem. J. A. Rees has selected the major papers in this field and has linked them with comments and an introduction. Reprinted from the the original sources, the papers together provide a unique introduction to the ionisation of gases.

#### Introduction to Space Charge **Electron Optics**

G. A. Nagy and M. Szilagyi

This introduction to space charge electron optics is an updated translation of an original Hungarian publication which presents to the West for the first time a comprehensive survey of the vast work carried out in this field in the USSR and Eastern Europe. It is however more than just a symmary of literature as it also contains the solution of many problems and so points the way to future developments.

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# GILSON

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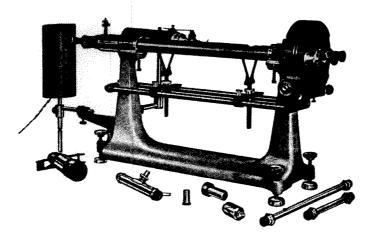
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#### **Biochemical Reactors**

B. Atkinson August 1974, 267 pp., £5.00/\$13.00

0.82086.042.3

The pressure created by demands for new products and processes necessitate rapid changes in the biochemical process industries. Continuous processes must replace batch processes, but require much higher levels of biochemical, microbiological and engineering knowledge. In this work the author, while dealing with fermenter and reactor design, develops the algebra necessary to describe the operation of "ideal" fermenters, where the fluid mechanics and the relations between substrate uptake and product formation are relatively simple.

London papers in regional science

### Space-Time Concepts in Urban and Regional Models

edited by E. L. Cripps August 1974, 237 pp., £4.00/\$10.25

0.85086.044.X

The papers in this volume, presented at the 1972 Annual Conference of the Regional Science Association, deal with aspects of the spatial organisation of cities and regions, and a considerable number of them deal with the development of spatial systems in time. The papers are organised into five parts; the notion of space-time development in regional science, developments in more conventional modes of regional economies, abstract models relying mostly on theory and analytical methods for their solution, the development of more operational models and the methodology of urban design and planning.

Pion applied physics series

### **Introduction to Noise Analysis**

R. W. Harris and T. L. Ledwidge September 1974, 102 pp., £2.50/\$6:50

0.85086.041.5

As on-line machines capable of rapidly analysing such statistical parameters as probability functions, spectral composition and correlation functions become available to practicing engineers and scientists, the application of no se techniques will increase. It was with this class of non-specialists in mind that this book was written. Noise analysis is not offered as a universal technique capable of solving all problems, but rather as an adjunct to the classical methods already well known and tried.

Pion advanced biochemistry series

### **Brain Function and Macromolecular Synthesis**

August 1974, 156 pp., £3.50/\$9.00

0.85086.043.1

In this book, Dr. Jacoubek brings together for the first time the vast range of observations relating brain function to the synthesis of macromolecules, and provides a critical interpretation of these observations in the light both of theoretical and methodical considerations. While placing emphasis on the difficulties met in measuring changes in the rates of macromolecular synthesis in nerve cells, he underlines the part played by stress reactions that are involved in a number of so-called learning

Pion monographs in spatial and environmental systems analysis

## Statistical Analysis of Spatial Dispersion

A. Rogers

October 1974, xii+164 pp., £4.50/\$11.75

0.85086.045.8

This book focuses on statistical methods for analysing the spatial distribution of a set of points within a circumscribed study area and applies such methods to an important problem in urban geography—the description and analysis of the spatial dispersion of retail establishments in urban areas, geographer, economists, marketing specialists, urban planners, demographers and sociologists will all find that the methods described in this book may easily be applied to spatial problems in their particular

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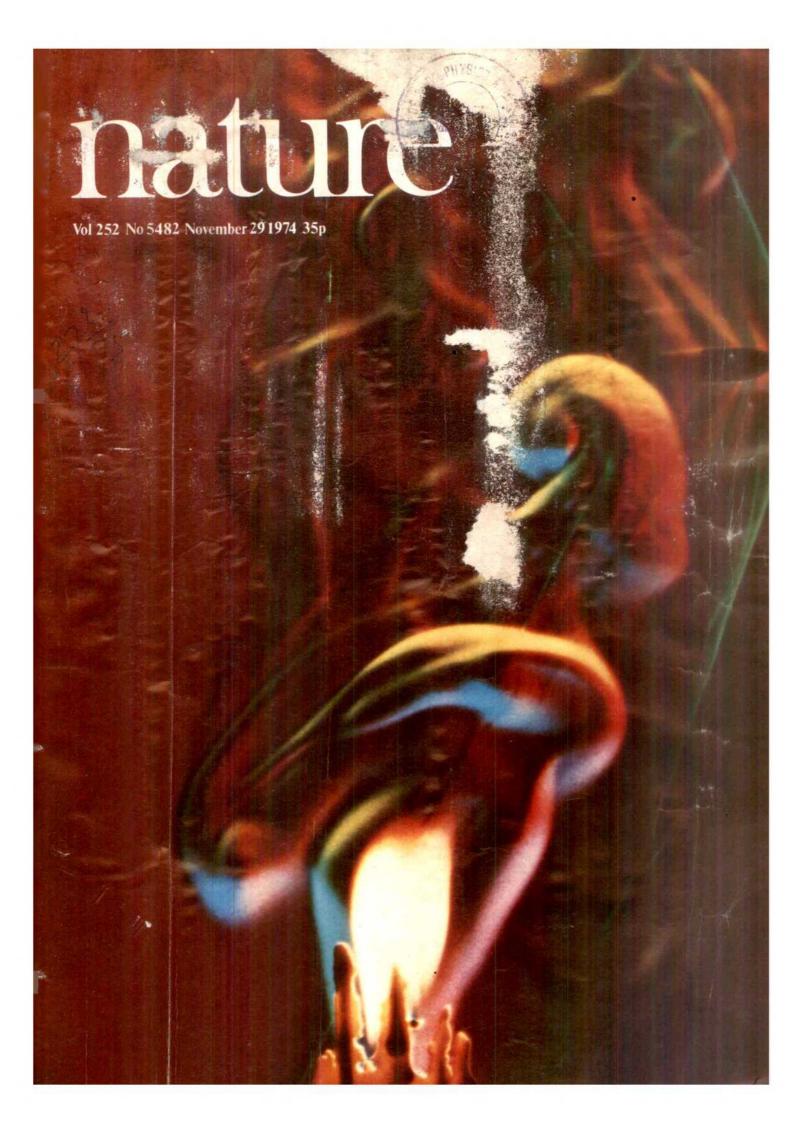
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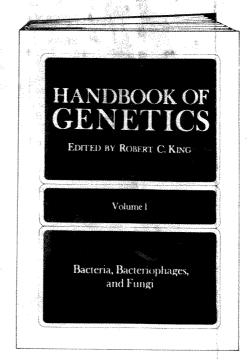


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Kim Vandiver writes on page 346 of recent developments in schlieren photography.

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#### Guide to authors

Nature accepts three types of communications:

- Articles are up to 3,000 words in length with at most six displayed items (figures and tables) and may either be reports of major research developments in a subject or broader reviews of progress.
- Letters are brief reports of research of unusual and wide interest, not in general longer than 1,000 words; at most they have three or four displayed items (figures and tables).
- 'Matters Arising' permits occasional short discussion of papers that have previously appeared in Nature. A limit of 300 words is placed on contributions.

Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the Système International. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible, exp (a) is preferred to e<sup>a</sup> if 'a' is more than one character. Articles should be accompanied by an abstract of not more than fifty words, and the abstract should list the main conclusions that are drawn.

References are indicated by superscripts in the text. See any contemporary Nature for style, but note:

- (i) To refer to several references by the same author at once, only one reference number need be given.
  - (ii) Cite first and last pages.

Abbreviations should follow the World List of Scientific Periodicals, fourth ed. (Butterworth, 1963-65). Symposia are often difficult to refer to and only published or soon-to-be-published volumes should be mentioned in references. Their publisher and place of publication should be clearly indicated. 'Personal communication' and 'unpublished work' should be incorporated in the text

Artwork should be sent with the manuscript. All artwork should be marked with the author's name. Line drawings should preferably be in Indian ink on heavy cartridge paper although other materials are acceptable; thin, shiny, folded, torn or heavily handled material should be avoided. Matt rather than glossy photographs are preferred. Figures are usually reduced to one column width. The originals should be about as wide as a page of *Nature*. Figures, particularly maps, should contain nothing but essential material. It is preferred that the original be unlabelled, but with a copy containing lettering. Labelling on photographs should if possible be avoided entirely. We are always glad to see artwork for possible use on the cover, whether or not it is associated with a manuscript, but cannot guarantee the return of material.

A fuller guide appeared in Nature (246, 238; 1973).

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"We would be happy to publish your letter if you could cut it in length by about 30%."

"We could publish your communication at letter length of about 1,000 words but not as a 3,000-word article."

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"We regret that your letter seemed too specialised for a journal aiming at a wide readership."

These are four of the types of letter that we don't like sending any more than you like receiving. For some years Nature has been publishing communications in the form of commissioned articles or reviews, research articles and letters. Looking back further, however, one sees that this format has only been reached by an evolutionary process; indeed the early issues of Nature contained an extraordinary diversity of material which the editor did little to segregate. Quarrels, opinions, anagrams and observations of atmospheric phenomena rubbed shoulders with announcements of scientific progress. Presumably those with a serious message did not have as great an objection as present-day scientists would to their scientific wheat sitting amongst anecdotal chaff—probably because they knew that next time they wrote it was as likely as not to be an anecdote that they sent.

In the past year we have tried to continue to evolve. A section has been started in which scientists can raise, briefly, points relating to earlier papers in Nature. (Though not too much earlier—about a year after publication we have to assume that ideas floated in our columns are starting to go through the normal refining process in the specialised literature and need no corrective action from us.) Further we occasionally take a more general communication and publish it in News and Views, particularly when it raises a controversial point of broad interest. Finally, we try to include after the leader a piece concerned with broader issues of science—policy, politics, method and so on. As often as not this will have been submitted originally as a letter. We are always pleased to see potential material both for News and Views and for the 'broader issues' pages, although the criteria by which we accept or decline have to be fairly subjective.

Although we have some instinct for what should go into these sections of the journal, and we expect that referees will have an instinct for the accuracy and general significance of the technical content of the papers communicated to us, we have a very limited ability to tell whether our present division into articles and letters and our present prescribed maximum lengths are in tune with authors' and readers' needs. Of course, we have (quite conflicting) opinions in the *Nature* office, and, of course, we do discuss the matter on occasions with scientists. But we lack any sort of market-place response. The number of subscribers who will cancel their subscription simply

because they find articles too specialised or letters too telegraphic is probably few. Again, it is difficult to judge whether a particular paper goes to another journal because it could not be condensed to a thousand words, because of the delay time in publication (though watch ours drop) or for any of half a dozen other reasons.

Because of all this, we take the perhaps foolish step of inviting opinions on how the medium could change the better to proclaim the message. There are some constraints. The upper limit of 3,000 words and six displayed items could not be revised upwards, simply because very long articles restrict the spread of interests in any particular issue and cut the acceptance rate for other manuscripts. Further, any policy by which the average length of letters is increased necessarily means that fewer can be published, as the number of pages that we can produce is unlikely to grow under present economic conditions. We do not, however, believe that the present acceptance rate of about 35% is an unalterable number.

Granted this, should we publish less or more papers of article length? And should we require that an article be particularly accessible to the general readership or would this destroy an important means of scientific communication? Should the distinction between articles and letters (a frequent bone of contention amongst authors cunningly submitting an 1,800-word paper) be abolished? Should the length limit for letters be raised—or even lowered?

We do make two pleas. First, that many authors have an ability to write to the limit whatever it may be and do not see it as a challenge to say in 600 words what might be expanded to 1,000. We shall continue to try to keep papers short and to cut out material which would be appropriate to a specialised journal but which gets in the way of the reader with limited time attempting to find out if there is anything in it for him. It is undoubtedly true that the shorter the paper the better the chance it has of being read widely.

Second, many papers have a depressing opacity which could easily have been alleviated before they were sent. If more authors would try out manuscripts on colleagues in different disciplines before submitting and would attempt to make the first paragraph into a crystal-clear description of what the paper is about rather than what other people's papers have been about, Nature would be an easier journal to read.



M. MARTIN, a French telegraphic engineer, has invented as engine for recording votes. The contrivance has been designed on the principle of the sonnettes electriques, and is exhibited in a shop in the Place Dauphine. The peculiarity is that the votes are registered and their total reckoned automatically. The invention is attracting public notice, as it is expected that the Versailles representatives will have an immense number of votes to register during the next session.

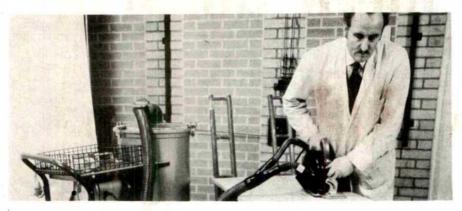
From Nature, 11, 94, December 3, 1874.

### For those in peril: 3 - not only on the factory floor

Dr D. R. Bowes of the Department of Geology at the University of Glasgow points out that not only workers but the general public could be at risk from asbestos and other mineral dusts in the environment and that more research is needed on this particular topic.

THE discussion by Peter J. Smith of occupational exposure to asbestos (Nature, October 18) highlights a major obstacle to a concerted effort by the scientific community in the field of the relationship of mineral and rock dusts to human disease, namely the apparent lack of awareness of the problems by many mineralogists, geochemists and geologists and hence of the neglect of their expertise. Some are involved but, however significant their contributions may be, it has been a relatively small group of the medical profession who has pinpointed the vital role of mineralogy and allied fields as well as demonstrated the health hazards associated with the inhalation of mineral and rock dusts. Much of the recent direction of public attention to the matter stems from an articulate few who see not only the value of scientific research to community health, but also to social and legislative action. They have been supported, particularly in the United States in recent years, by active collaboration with physical scientists, which has not only contributed to medicine but has opened frontiers in the physical sciences. In Britain it is more than 40 years since an industrial survey showed the high prevalence of asbestosis in asbestos factory textile workers, and that came nearly a quarter of a century after Parliament was first notified, in 1906, about fatal pulmonary asbestosis amongst asbestos workers. With such a passage of time, and its implications for human disease and suffering, the focusing of attention of physical scientists on occupational exposure to asbestos, and to the many other mineral and rock dust materials associated with mining, milling and manufacturing industry, is a matter of urgency.

Urgent attention should also be given to potential health hazards concomitant with environmental exposure. The incidence of pleural calcification resulting from environmental exposure in the neighbourhood of an asbestos mine or mill, or of pleural mesothelioma, without occupational exposure, in areas where there is asbestos in the local rocks, serves to illustrate that potential



Not the only one at risk

risk is not limited to occupationally exposed miners and industrial workers. The 1971 Report of the National Institute of Occupational Diseases, Johannesburg records that more than a third (of 210) of cases of mesothelioma were considered to be the result of environmental and not occupational exposure asbestos. This, together with evidence that asbestos greatly multiplies the already considerable lung cancer potential of cigarette smoking, suggests that combined scientific and medical research as a basis for legislative attention, as well as preventitive medicine, could have a significant effect on community health in at least some industrial areas.

The dimensions of any health hazard associated with air pollution caused by dusts, including asbestos, have yet to be defined and it is not proper to equate neighbourhood contamination with general community air pollution. The existence of levels of 10 to 50 ×10<sup>-9</sup> g m<sup>-3</sup> of chrysotile asbestos in the ambient air of places like New York City and the regularity with which asbestos fibrils are found at autopsy in the lungs of residents of New York and London, are probably indicators for other urban areas. The asbestos industry has an important responsibility for controlling the commercial and industrial sources of air pollution, but additional possible contributory factors, resulting from the action of the public at large, must not be passed by. These include dusts from asbestos brake drum linings and the mineralogy of decomposed lining material which could have a general effect as well as a localised one in the confined spaces of motor repair premises; inorganic particles in smoke from tobacco-that from some cigars is known to contain an inorganic fibrogen and that from cigarettes, is suspect in view of the relationship between cigarette smoking and lung cancer; and consumer talc products, which must represent one of the mineral dusts most inhaled and ingested by people of all ages in many households, with talc known to cause pneumoconiosis as the result of occupational exposure and to occur in nature together with some asbestiform minerals.

There are other factors to be considered in the control of environmental exposure to mineral and rock dusts through air pollution, and many other geological and mineralogical aspects to be assessed in the relationship between human health and the inhalation and ingestion of inorganic particles. And those instanced now under joint or separate investigation in the Environmental Science Laboratory, Mount Sinai Hospital, New York, and the Department of Geology, University of Glasgow show not only how in science something that is apparently obvious, with the benefit of hindsight, is not recognised until its possible effect on human welfare is appreciated, but also how in developing fields the availability of equipment that can help meet the new demands is vital. Identification of gaps in existing knowledge as suggested by Peter J. Smith is a useful next step but consideration of environmental exposure to mineral and rock dusts, as well as occupational exposure, is likely to reveal larger gaps than initially anticipated and to suggest that inclusion of matters affecting the health of the public at large will entail interdisciplinary endeavour on a considerable scale. Review and assessment alone will, however, not avert human suffering but could lead to frustration on the part of scientists and cynicism on the part of the general public if the will to accomplish is not matched by adequate provision of the tools needed, even if this means that certain other research topics, with less application to human suffering, are given reduced support.

# international news

THE European Science Foundation took a large step forward in Strasbourg last week with the election of officers and the creation of an executive council. Sir Brian Flowers, physicist and Rector of Imperial College, was elected President and the two Vice-Presidents are Professor O. Reverdin, a Greek scholar and Chairman of the Swiss Fond National and Dr P. Riis, a Danish physician. Secretary-General of the ESF will be Dr F. Schneider, at present Secretary-General to the Max-Planck Institut in Munich. Dr Schneider will work part-time in Strasbourg from January 1975 and eventually will be employed full-time by the foundation. Other officers will attend in Strasbourg for a couple of days a month on average, particularly for the meetings of the council of eighteen members. Britain has a second member on the council, Dr S. G. Owen of the Medical Research Council.

The ESF emerged as a concept when it became clear that there was considerable resistance to the idea of a pure research organisation being based on the EEC. Two of the most commonly heard arguments were that an ESF based on the Nine would be tied by the ponderous political procedures of Brussels and that learning knew no frontiers. Accordingly, and with the EEC's blessing, responsibility for the encouragement of European collaboration in the furtherance of research was passed on to an ESF comprised of representatives of almost all Western European countries, and there was a clear requirement that the body should have flexibility.

The word 'Science' in the title is interpreted in its broader European context of learning or knowledge. The 43 member organisations which sit in the assembly, the governing body of the ESF, thus comprise not only funding agencies such as research councils of the natural and medical sciences but also academies both of science and the humanities. The British seats in the assembly are occupied by the five research councils, the Royal Society and the British Academy.

The budget for the first year is to be 2.2 million French francs, most of which will go in administrative expenditures in Strasbourg; it is not envisaged that the ESF will become a funding agency. Rather it is seen as an enabling organisation, providing a forum for in-

# Flowers to head new European Science Foundation

ternational discussions on collaborative projects. There is to be no requirement that all members should agree on these projects; on occasions the ESF may simply serve as a template on which two or three research organisations in different countries can put together a scheme for which they have funds.

The success of ESF will clearly depend on its ability to show its useful-

Sir Brian Flowers



ness within the first year or two, and Sir Brian already has a list of projects to which the council will give early consideration. These are:—

Astronomy This is seen as a top priority as it is a natural field for multi-lateral collaboration and any consequent trimming of large national budgets on astronomy would be a big plus for the young ESF.

Archaeological techniques Clearly the archaeology of various parts of Europe differs widely, but techniques are much the same everywhere. The pooling of equipment and facilities could be the first step into the humanities for the ESF.

Comparative legal systems Getting to know how different countries work is going to be part of the European experience of the next ten or twenty years.

Social responsibility in biology Does this have a European dimension? ESF as yet does not know, but EMBO will be consulted early on the plasmid engineering issue.

Outside comment on large institutions Sir Brian would like to see ESF playing the same role in Europe as the National Academy of Sciences has done in the United States in providing informed comment and criticism on organisations such as ESRO. This is bound to be a fairly controversial proposal and he has already been presumed by some to be seeking to get at ESRO. Does ESF yet have the weight for this sort of operation?

Marine biology Ireland is particularly keen to advance in this field.

Science policy There is thought to be scope within the ESF for discussions on the development of a more pan-European approach to policy-making.

Supporters of the ESF are at pains to point out that one of the first jobs of the foundation is to establish good working relations with the already-existing European-wide science and medical research council collaborative efforts. Since it is these councils at a national level which have been, interalios, responsible for setting up the ESF this should not be a major difficulty.

In the longer run the ESF is seen as a body which national governments and international organisations will consult as a matter of course, if not as a legal necessity, on major matters of science and learning.

### New Astronomer Royal for Scotland

by John Gribbin

THE announcement that Dr V. C. Reddish is to succeed Professor H. Brück as Astronomer Royal for Scotland and Director of the Royal Observatory, Edinburgh (ROE) comes as no surprise to astronomers. Dr Reddish's new post is one of the two key jobs in the rebuilding of British optical astronomy (see Nature, 251, 456; 1974). The Royal Greenwich Observatory (RGO), under the Directorship of Professor F. G. Smith, will have responsibility for running the new Northern Hemisphere Observatory, whereas the ROE provides the base in the United Kingdom for the Science Research Council (SRC) team running the 48inch Schmidt Camera at Siding Spring and also plays a large part in the Anglo-Australian Telescope project. In addition, the ROE has the responsibility of setting up the 3.8-m infrared flux collector which is to be built in Hawaii with SRC funds (see Nature, 250, 617; 1974). So the responsibility for the hardware of what Professor Smith sees as a new 'Golden Age' of astronomy is divided evenly between Britain's two principal optical observatories.

Like Professor Smith at the RGO, Dr Reddish seems well qualified for the



task. His experience is rather more diverse that that of most academics, embracing 12 months in a brewery, three years in a bank and three years in the Royal Navy before he took his first degree. Since gaining his PhD as a member of University College, London, in 1954 Dr Reddish has been based in Edinburgh except for three years from 1959, which he spent at Jodrell Bank.

He says that those three years were

of great importance since they introduced him to the teamwork approach used by radio astronomers; with the electronic equipment attached to telescopes becoming ever more sophisticated there is now very little difference between the tasks of observers at radio, optical and other frequencies, and Dr Reddish realised from his experience at Jodrell Bank that modern astronomers would have to follow the same path of collaboration in order to get the best results out of their new instruments.

Since 1962 Dr Reddish has worked from time to time as a visitor at Hamburg, in the United States and in Australia; he was Project Officer for the construction of the United Kingdom Schmidt in Australia, and serves at present as one of three British members on the AAT Board. It is interesting that the future of British optical astronomy is largely in the hands of one man who has 'converted' from radio astronomy, and another who says that he learnt a great deal from his three years at Jodrell Bank. But divisions between observers today mean very little, and the real importance of both these appointments is that the men concerned have the proven administrative experience, as well as appropriate scientific pedigrees, to ensure that full advantage is taken of the opportunity now presented to British astronomy.

THE Soviet Mars mission of 1973-74 was only a partial success, with one intended satellite (Mars 4) and one landing craft (Mars 7) overshooting the planet. Results of the data from the more successful probes (Zemlya i Vselennaya, No. 5, 7-11; 1974), although scientifically stimulating, may well give certain embarrassment to Soviet politicians, for—in spite of their predilections for the colour—the red planet is, in fact, not quite as red as it has previously been painted.

According to the colour photographs received from the orbiting Mars 5 (and from the overshooting Mars 4), the craters in particular reveal significant variations of colour. Some of them exhibit a blue-green tint, contrasting sharply with the general orange background of the crater bottom. "If the correctness of the colour transmission is confirmed", says the report with some caution. "this may indicate that the bottoms of these craters are composed of rock different from that on the surface". Or (even more cautiously) "maybe, after all, there still exists vegetation on Mars?"

Passing quickly from these speculations, however, the report states a number of quantitative facts: the

### The not so red planet

from Vera Rich

argon content of the Martian atmosphere (according to mass spectroscopy measurements from Mars 6) has the very high value of  $35 \pm 10\%$ —a result at present inexplicable, although relative argon enrichment of the atmosphere as a result of the freezing of  $CO_2$  to form the polar caps has been postulated tentatively.

The amount of water vapour in the Martian atmosphere was found to be much higher than measured by Mars 3 and at places the amount of precipitated water reached 60-70 µm, some 3-5 times higher than the values obtained from the Mars 3 data of 2 years ago. The distribution of this moisture over the surface of the planet was found to be irregular. The joint Franco-Soviet polarimetry experiment gave results far lower than expected, the greatest polarisation (up to 10%—some 2-3 times less than that of the Moon)—being observed close to the northern extremity of the Argyre crater sea.

Infrared measurements gave surface temperatures very close to the theoretical models for highly fragmented rock; the highest temperature recorded being 272 K, corresponding to 1300 h local time. Correlation of surface temperature and relief has still not been observed.

Photography of the topographical features, apart from the curious problem of colour, has produced interesting results. With the high resolution used (down to tenths of a kilometre), the existence of two forms of crater was established: large craters having a flat bottom and slopes considerably affected by wind erosion, and small cup-shaped craters.

For a mission only partially successful in its hardware, the Soviet Mars survey of 1973-74 has not been unrewarding. Perhaps, however, the most significant data recorded are those from the analysis of the phase variation of the radio signals from the probes. From this it has been estimated that the electron concentration in the Martian ionosphere is  $6 \times 10^3$  electrons per centimetre at night and 1.5 × 105 electrons per centimetre by day. It seems likely that on the basis of these figures, future Mars probes will use wavelengths longer than 500-1,000 m for radio communication.

THE Think Tank's recent review of energy conservation possibilities (see Nature, July 5) is the subject of five weekly meetings at the Royal Institution where some of the propositions and recommendations put forward in the report will be discussed and criticised. Last week's meeting dealt with the potential of tidal power—on which the report was unenthusiastic—with a quick look at wave power, regarded more favourably by the Think Tank.

The first point stressed by the speakers, Mr David Gwynn and Mr Brian Severn of Engineering and Power Development Consultants—a member of the Balfour Beattie Group—was that tidal power does work and that there is experience available in facing and overcoming the technical problems which arise.

Nobody claims that tidal power is the answer to Britain's energy problem. A scheme in the Severn Estuary, for instance, could provide a maximum of 4.5 GW and would save about 5 million tonnes coal equivalent each year in fossil fuels. But it is an inexhaustible resource and, even on the limited scale that would be possible in Britain, could produce a useful minor diversification of the energy base as part of an integrated power generating system.

#### There is a tide . . . .

The disadvantages of tidal power when viewed simply as an isolated source of electric power generation are to some extent mitigated when it is considered as part of a total energy system. The snags are, of course, that power generation is irregular. In any period of 25 hours there will be four periods of slack water when power cannot be generated and the range of tides also varies considerably.

The possible role of tidal power in providing a source of heat for district heating would be one way of overcoming the disadvantages of irregular power generation. Mr Gwynn and Mr Severn also see a role for tidal power in smaller local projects (not necessarily in Britain) in integrated schemes for estuary basins involving water storage, and in recreation (yachting, for example) with a small tidal power station serving some local use such as pumping water for irrigation.

One of the assumptions in the Think Tank report was that water storage and power generation in the same estuary would be incompatible. This was challenged by Mr Gwynn who pointed out that limited freshwater storage in 'bunded' reservoirs would not interfere with tidal movement. Larger schemes

for barrages across the mouth of the estuaries for water storage are now to some extent out of favour.

Compared with tidal power, the practical capabilities of wave power are as yet untried. And there are still many unsolved problems such as storing the power generated and transmitting it to the shore, quite apart from the possible technical difficulties in manufacturing the massive steel structures which are needed for the most promising scheme involving wave power.

But in the long term wave power could theoretically provide an inexhaustible source of almost all Britain's energy requirements, and is the one new 'unconventional' source of power singled out by the energy conservation report as worthy of serious investment and consideration.

Some wider implications of the use of wave power were touched on by the meeting chairman, Professor John Page of the University of Sheffield. What effects, he wondered, would the presence of strings of these massive structures out in the Atlantic have on overall wave movement and what could be the consequences to coastal ecology and fisheries? Also, in the present confusion and uncertainty over the rights of nation states over the oceans, would there also be objections on legal grounds?

# correspondence

#### **Bacterial** engineering

SIR.—The anxiety expressed by various scientists on the hazards of partially hybridising certain types of microorganisms has already led to considerable public disagreement. It is clear that some responsible and established workers consider it unwise to take humanity with them on any tour into the unknown, while others consider it impractical to curtail the spread of organisms by any method proof against technical errors, psychoses, and even earthquakes. This will remain true whatever is stated by any of the many committees which are likely to advise each advanced nation.

I wish to advance a simple positive proposal which cannot fail to increase the safety of any bacterial incorporation studies, and which would itself provide interesting examples of extreme adaptation which might have applications to other fields, such as waste disposal.

Contracts should be offered for the development of an organism, with suitable qualities for work on partial

hybridity, which would not grow within a pH range of 6–8, would need an oxygen partial pressure of at least three-fold the normal, and which was dependent on at least two unusual synthetic substrates. These should be very simple safeguards against any single mutation allowing reproduction in any natural host.

Once such an organism was established, it should only be necessary to provide substantial subsidies to firms undertaking to provide the necessary culture media—and such media could hardly be misappropriated—to provide an economic and technical climate within which temptations to continue to work on the worst possible organism—a commensal of the human gut—would be seen as both unnecessary and irresponsible.

Since even bacteria take time, and moratoria cannot be expected to contain curiosity for long unless alternative outlets are provided, and seen to be imminent, the matter would seem urgent, and probably too urgent for the attention of the present public funding

organisations. Even if no immediate funds are available from such sources informed discussion might influence some companies which supply media and equipment for tissue culture to initiate work in the hope of reaping the large commercial rewards such developments could yield.

J. H. EDWARDS Queen Elizabeth Medical Centre,

Why then publish?

Birmingham, UK

SIR,—Recently I was a co-author on a paper in *Biochim. biophys. Acta*. Of the 140 reprint requests addressed to me no less than 70.5% were addressed to an unknown Dr B. R. Carter. I hope this frequent oversight is not a reflection on the powers of observation of the modern scientist. Perhaps this is one way of discouraging people from publishing too many papers: deny them the right of recognition.

Yours faithfully,

B. R. CATER

Sheffield, UK

SIR,—It is regrettable that your journal should have given such prominence to an article-"Doubts Over US in India" (Nature, September 20)-consisting of vague allegations and innuendoes against respected scientific bodies. In the entire article I found only three factual statements capable of verification (the others being matters of opinion) and these are quoted from a letter to a newspaper. For the rest, the author seems to rely on unnamed "Indian scientists" or "experts". No names or designations are given and it is difficult to place much value on such anonymous allegations.

I would like to state the facts about the Bombay Natural History Society and its collaborative research with the World Health Organisation (WHO) and other organisations, with which I have been personally connected, so that your readers have an opportunity to form their own judgement of the matter.

In 1957 a 'new' virus disease was detected in Mysore State. This Kyasanur Forest Disease (KFD) virus closely resembled the virus of Russian Spring Summer Encephalitis and raised the possibility that the disease was brought to India from Siberia by arthropod vectors carried by migrating birds. A collaborative study of the problem was organised by the WHO, under Dr Salim Ali as principal investigator, in which the Bombay Natural History Society studied the migratory birds and the Virus Research Centre, Poona, carried out the virological studies on the birds and their parasites. After some time, it seemed that transmission of disease by this route, though possible, was not a significant epidemiological or public health problem. The WHO, accordingly, discontinued its support of the programme. The Bombay Natural History Society continued to work, although no virological studies were possible since neither the Virus Research Centre nor the Haffkine Institute (where I was then Head of the Department of Virology) could spare the necessary staff for a study. For • a time, at the instance of the WHO, blood samples were sent to the Institute of Diseases with Natural Foci at Omsk, since many migrant birds come to India from that area.

At about this time the United States Army Migratory Animal Pathological Survey (MAPS) organisation was studying migratory animals and a proposal for continuing the bird migration study in collaboration with them was accepted with the approval of the Indian Ministry of Defence. No American scientist was designated for this programme by the MAPS. In addition to the migration studies, blood smears from the birds were collected and, at the outset, were sent to the

MAPS for study. Such slides could have been provided to any other agency interested. After a while the MAPS discontinued this study and several hundred slides are still at the society awaiting study by some interested party.

Subsequently grants under PL480 funds were made to the Bombay Natural History Society in collaboration with the Smithsonian Institution for a study of bird migration, and this continued until 1973 when all PL480 grants were stopped by the Indian government. These grants are made for

# Answer from India

from A. N. D. Nanavati, Bombay Natural History Society

collaborative research programmes approved by both the United States and India, and are operated by scientists from both countries. The Indian government does not accept any such research programme without careful scrutiny by independent experts. There are hopes that the grants for this study may be revived.

The main point on which suspicion has been focussed is the collaborative study with the MAPS, because of the fear that information so obtained may be useful in biological warfare. So long as there is no secrecy involved, there can be no objection to any research, otherwise we would be constrained to ban every textbook on epidemiology and communicable diseases. This research programme was open and the information was available to anybody interested. The bird camps were visited by ornithologists and other scientists from several countries (including workers from the Institute of Diseases with Natural Foci, Omsk) all of whom had full and free discussions on the information collected. Much has also been made of the proviso that the MAPS

required that no material be published without clearance from it. There was no such provision in our contract, merely that the MAPS should receive two advance copies of any publication or report. With this information, the only factual charges made by 'a professor' and quoted by Mr Sehgal can easily be disposed of:—

- (1) That blood smears were sent to the MAPS for examination. They could also have been and can still be made available to any other interested party.
- (2) That two US scientists from the Smithsonian Institution were associated with the Bombay Natural History Society. One scientist, Dr Dillon Ripley was designated as the American Investigator and Dr Salim Ali was the Chief Investigator from India. This was part of the routine in any PL480 financed study.
- (3) The services of the Bombay Natural History Society and the Virus Research Centre were made available to United States Army personnel for a nominal fee. This seems to be a figment of someone's imagination. Neither the Bombay Natural History Society nor the Virus Research Centre are commercial organisations. The Bombay Natural History Society offers assistance to any serious student, wherever he comes from. No charge is made for such assistance.
- (4) That only partial results were available to the Indian participants. We have, in India, people of sufficient experience in epidemiology and microbiology to be able to assess the significance of these studies and to detect whether there were any significant omissions or false information in the results supplied. This charge is, however, a matter of opinion which could only be resolved if Mr Sehgal would disclose some of the information which he claims has been withheld.

It seems then that the gravamen of Mr Sehgal's charges is that all the Indian scientists working on collaborative research programmes with international or foreign agencies are gullible and will believe whatever their collaborators tell them. He therefore ignores the considered statement made by our President, Dr Salim Ali, but would place his faith in the rumours and 'feelings' of anonymous authorities, unsupported by a single verifiable factual statement. Can it be that there is no monopoly on gullibility?

This is not to suggest that we can afford to be careless. Dangers do exist and must be carefully guarded against. It is unfortunate that in trying to highlight possible dangers Mr Sehgal has cast aspersions on institutions and persons who are fully aware of their responsibility and have taken all possible safeguards against such dangers.

# news and views

### No consensus yet on climate

In this issue of *Nature* (pages 368 and 370) is another instalment of the saga in which non-meteorologists suggest to the professionals processes, hitherto unrepresented, that may account for their lack of forecasting skill. The meteorologists' usual response is, as Sawyer's here, that it is not dubious new processes that are required but better understanding of those already known to be important.

Meteorologists are acutely aware that their data are so abundant that fortuitous associations of some of them with unrelated phenomena are bound to exist. To some extent King's scholarly conjectures about the association of magnetic field and climate are liable to this reservation. Sawyer shows, moreover, that there has been some selection of data in King's quoted evidence—the association he describes is much better for latitude 60° N in the winter than for other latitudes and seasons.

But I was still a little surprised at Sawyer's comment which is based on the fidelity of current numerical models of the atmosphere and in particular with his degree of confidence in what is, after all, one of the less sophisticated models of global-scale processes. To my mind this is deftly and succinctly qualified by King's reply, and one should indeed bear in mind a further qualification. The numerical models of atmospheric circulation do not represent quite such pure physics as might be supposed at first sight, for they have to be 'tuned'. By this process values of parameters appearing in the models are adjusted to make the model behave more realistically such processes as surface friction, the transfer of energy by electromagnetic radiation and many cloud effects usually have to be treated in this way. Although this is a legitimate way of establishing a mutually consistent set of interacting processes, there is clearly room for King's as yet unidentified process.

The evidence for an association of geomagnetic and meteorological patterns presented by King is tempting (cor-

relations of 0.96 are not to be sneezed at). Causal connection is a remote possibility awaiting a plausible quantifiable hypothesis that is not even remotely hinted at by King. His speculation that "Coriolis force, inertia and viscosity of the air" may account for some differences seems naive. Meteorologists know about such things and have taken them into account in many elegant and detailed treatments which show how the atmosphere responds to any forcing, whether by anomalous heat sources, undulation of surface elevation or indeed ill-defined but energetically small magnetic effects. Such analysis shows that motion of large scale penetrates upwards high into the atmosphere, where the energy is partially reflected downwards. Such reflection is governed by the large-scale properties of the upper layerslike the general wind and temperature structure, which may indeed be conditioned by some much less intense energy sources.

Given finally that the large scale systems may be close to resonance (particularly in the winter) we have the beginnings of some process by which climate may be modified by weak energy sources, especially those in the upper atmosphere but, it must be stressed, dependent not on local anomalies of energy but on variability of the global scale circulation.

If causal effects of one on the other are unlikely, is it possible that both climate and magnetic field perturbations have a common origin? It is widely believed that the details of the geomagnetic field originate at the core-mantle boundary, if not in the core itself—an unlikely spot, one would think, for climate to be controlled from. But the obvious influence of continents on climate and the less obvious influence of continents on upper-mantle resistivity profiles could, just conceivably, be a link—the upper mantle being a window through which core effects are seen.

J. S. A. GREEN

### The self control of myosin

Two recent papers have reopened the debate about the existence of a Ca<sup>2+</sup>-sensitising mechanism associated with the thick filaments in vertebrate muscle.

By the late 1960s it was generally believed that contraction of vertebrate skeletal muscle—and by assumption also of other muscles—was triggered solely by the binding of Ca<sup>2+</sup> ions to the troponin components of the thin filaments. The evidence for this was that troponin was apparently the only myofibrillar protein which could reversibly bind Ca<sup>2+</sup> in the presence of Mg<sup>2+</sup> ions and that troponin, together with tropomyosin, was required to confer Ca<sup>2+</sup> sensitivity on the ATPase of vertebrate skeletal actomyosin.

The idea that all muscles were controlled through the thin filaments in this way was scotched by a series of papers by Szent-Györgyi, Kendrick-Jones and Lehman. They were able to show that whereas some invertebrate muscles had this troponin control, others (most notably certain molluscan muscles like the cross-striated adductor muscle of *Pecten*) lacked troponin but instead contained a special

kind of myosin which could bind Ca<sup>2+</sup> in the presence of Mg<sup>2+</sup> ions. Correspondingly, the actin-stimulated myosin ATPase from such muscles was Ca<sup>2+</sup> sensitive. In other words, such muscles were controlled through the myosin of their thick filaments. Yet other invertebrate muscles had both forms of control. The test devised to determine which kind of control is present in any given muscle is simple. The effect of adding pure actin to the contractile apparatus is observed. If the Ca<sup>2+</sup> sensitivity (that is the ratio of the ATPase activity in the presence and absence of Ca<sup>2+</sup> ions) is reduced, then only the troponin control must be present since the thin filament activity has been swamped by the excess unregulated actin. But if the sensitivity is unaffected there must be myosin control.

Szent-Györgyi et al. examined both smooth and striated muscles of invertebrates but only the striated muscles of vertebrates. Now Bremel (this issue of Nature, page 405) has applied their test to a vertebrate smooth muscle (chicken gizzard), about whose control little is known. The

addition of pure actin has no effect on the Ca<sup>2+</sup> sensitivity of a crude preparation of chicken gizzard actomyosin. This shows that a myosin control is present. To see whether or not troponin control is also present, Bremel has applied the complementary test. The addition of rabbit heavy meromyosin to the preparation reduced the Ca<sup>2+</sup> sensitivity. When due allowance is made for the activity of the chicken gizzard myosin, it turns out that the added heavy meromyosin ATPase (stimulated of course, by the chicken gizzard actin) is not Ca<sup>2+</sup> sensitive. Bremel concludes that vertebrate smooth muscle lacks troponin and is analogous to those invertebrate muscles which have only myosin-linked calcium regulation.

Even in vertebrate skeletal muscle there is growing evidence for a thick filament control. A few years ago, Haselgrove and H. E. Huxley examined by X-ray diffraction muscles stretched to lengths where no overlap would be expected between thick and thin filaments. Nevertheless on stimulation of the muscle, movements of the myosin heads, recognised by a loss of their helical order, could be observed. The simplest interpretation was that the thick filaments of vertebrate skeletal muscle were themselves directly responsive to Ca<sup>2+</sup> ions. Possibly, however, some overlap of filaments was still present in local regions of the fibre and changes in the region of overlap were propagated to the rest of the thick filaments. Because there was this doubt, the significance of these important experiments was not immediately felt.

A change of thick filament conformation should affect its sedimentation coefficient. So Morimoto and Harrington (J. molec. Biol., 88, 693; 1974) have examined the rate of sedimentation of isolated natural thick filaments in the presence and absence of Ca<sup>2+</sup>. A small but significant increase in the sedimentation coefficient with rise of Ca<sup>2+</sup> concentration is indeed observed. The midpoint of the transition is at a Ca<sup>2+</sup> concentration of about 3×10<sup>-6</sup> M (well below the level of Ca<sup>2+</sup> thought to be reached inside an activated muscle fibre) and is rather insensitive to the Mg<sup>2+</sup> concentration. Hydrodynamic theory is unfortunately too complicated to say exactly what changes in conformation are responsible for the variation in sedimentation coefficient.

Similar experiments with synthetic thick filaments prepared from purified myosin establish that myosin, rather than C-protein, is the  $Ca^{2+}$ -sensitive protein. This was surprising since it has been assumed that vertebrate skeletal myosin binds little  $Ca^{2+}$  in the presence of concentrations of competing  $Mg^{2+}$  ions similar to that in muscle ( $\approx 1 \text{ mM}$ ).

Morimoto and Harrington have gone on to redetermine the Ca<sup>2+</sup> binding properties of myosin in the presence of Mg<sup>2+</sup>. They find that each myosin molecule has two Ca<sup>2+</sup> binding sites with an affinity accounting for the sedimentation data. The sites turn out to be located on the light chains removable by 5,5' dithiobis-dinitrobenzoic acid (DTNB). It remains to be seen how the binding of Ca2+ ions to these chains could produce large-scale movements of the myosin heads. It is just possible that the DNTB light chains are not associated with the heads themselves (as is generally thought) but rather with the 'necks' joining the heads to the tail of the molecule. In this position they could perhaps influence the movements of the heads. The point of special interest is that the DTNB light chains of vertebrate skeletal myosin were recently shown to be capable of substituting for the Ca2+-sensitising light chains of Pecten myosin (see Nature, 249, 609; 1974) which suggests a considerable similarity between the two kinds of myosin. Nevertheless, the temptation to think of the myosin control in vertebrate skeletal muscle as being entirely analogous to that in invertebrate muscle should be resisted for the moment. There remains an important difference to explain: the actomyosin ATPase of vertebrate skeletal muscle is not sensitive to Ca2+ ions, whereas that of Pecten actomyosin is. In other words evidence is lacking that the regulation of cross-bridge movement by Ca<sup>2+</sup> affecs ATP consumption. Whether this difference is attributible to a greater lack of spatial organisation of the verterate filaments in experiments conducted in the test tube remains to be seen. We can certainly expect a spate of papers probing this problem.

The conclusion that in vertebrate skeltal muscle there is control through the thick filaments as well as the thin filaments is attractive. It always seemed priverse of nature to control cross-bridge activity through the thin filaments when a more obvious form of control would be to regulate the movement of cross-bridges directly. The existence of two Ca<sup>2+</sup> sensitising mechanisms, one on each kind of filament in vertebrate skeletal muscle, would presumably increase the sensitivity of the contractile apparatus to Ca<sup>2+</sup> ion concentration and ensure that the breatdown of ATP in resting muscle was kept at a low leve. The relative importance of the two forms of control remains to be assessed.

# More false hopes about solar neutrinos

One of the principle activities of stellar astrophysicists in the last five or six years has been to tackle the solar neutrino puzzle. Since Davis and his collaborators announced an upper bound to the flux of neutrinos incident on the earth from all sources which was only one tenth the value theorists had predicted from the Sun alone, astrophysicists have rightly been concerned about their understanding of the Sun. Initially there was a massive reappraisal of the theory, and in particular of the nuclear physics needed for computing the rates of the energy and neutrino producing reactions in the solar core. Improvements were made both to the equations governing the solar models and to the numerical methods used to solve them. But on the whole these did not change the theoretical solar neutrino flux by nearly as much as was necessary.

Perhaps the most profitable activity was to adjust certain inaccurately known parameters, such as those defining the initial conditions. This led to the construction of what has become known as the 'standard model': namely, that solar model with all such parameters adjusted to the limits of plausibility in such a direction as to minimise the predicted neutrino flux. There are now several standard models, independently computed by different workers, and all yield similar neutrino fluxes rather lower than the pre-Davis values. As to the state of the measurements, Davis has refined his techniques and now quotes an upper bound which, in round numbers, is still about a factor ten below the theoretical values.

The recognition that standard models would probably never fit the observations has provoked much questioning of the premises from which they have been constructed. Most of the papers published are of an exploratory nature, asking merely the question: "How can the theoretical neutrino flux be reduced below Davis's limit simply by arbitrarily though not entirely implausibly adjusting the unconfirmed assumptions of the theory?" A complete answer to that question would be a very valuable first step towards the solution of the puzzle.

One line of investigation has been to postulate that the inside of the Sun is rotating much faster than the surface. Early calculations along these lines were unsuccessful, but recently Demarque, Mengel and Sweigart (Astrophys. J., 183, 997; 1973; Nature phys. Sci., 246, 33; 1973) and Roxburgh (Nature, 248, 209; 1974) have produced models which they thought predicted neutrino fluxes below the observed

flux bound. These models have extremely rapidly rotating cores with the ratio  $\lambda$  of centrifugal to gravitational forces being as high as  $\frac{1}{4}$  to  $\frac{1}{2}$ .

But a serious flaw in the calculations has recently been pointed out by Monaghan (Mon. Not. R. astr. Soc., **169**, 13P; 1974) The rapidly rotating models were computed under the assumption that \( \lambda \) is very small, and approximations essentially equivalent to using  $1 + n\alpha\lambda$  for  $(1 + \alpha\lambda)^n$ , where  $\alpha$ is of order unity, were used. In the calculations of the neutrino flux n is typically between 25 and 30, and for such values, Monaghan points out, the approximation leads to gross underestimates when  $\lambda$  is as large as Demarque et al. and Roxburgh require, whatever the sign of  $\alpha$ . It seems, therefore, that even allowing for the errors arising from this approximation in the calculation of the mean energy generation rates, the quoted theoretical neutrino fluxes have been underestimated by at least a factor three.

A second criticism is raised in today's issue of *Nature*. Rood and Ulrich (page 366) have repeated the calculations

of Demarque et al. and have argued that the latter authors made a mistake in their computation of the oblateness of their models. This is admitted by Demarque et al. on page 368. When the mistake is put right, the oblateness comes out to be greater than the measured value that R. H. Dicke reports. Roxburgh's model is not ruled out by Dicke's measurements, but it is contradicted by the recently announced results of the more careful investigations by H. A. Hill and his collaborators, which imply an almost spherical Sun consistent only with a rotation of the interior hardly faster than that at the surface.

Rood and Ulrich go on to investigate the combined effect of rapid rotation and a postulated transient convective mixing triggered by shear turbulence. Of course this raises the obvious difficulty of justifying why the Sun is in a unique peculiar transient state at the present time; but in any case the models presented today fail to yield sufficiently low neutrino fluxes combined with an adequately small oblateness, even though they are based on

the small  $\lambda$  approximation and are subject to Monaghan's criticism. Rood and Ulrich conclude that rapid rotation is not a clue to the solution of the neutrino puzzle. They are probably right.

D. O. Govon

# Inbreeding to preserve diversity

from our Animal Ecology Correspondent In the Uganda kob antelope there exists a system of social behaviour clandestine to prevent designed matings. Individual males fight for and guard small territories of between 10 and 20 m in diameter. A cluster of 30 to 40 such territories is called a lek. In the Torro Game Reserve in Western Uganda there are 18 such leks in an area of 400 km2. Courtship and mating take place within the territories amid a complex ritualised dressage which seems to be designed as if to publicise the fact that a mating has taken place. The details of the lek behaviour are well known (Buechner, Science, 133, 698; 1961; Proc 16th int. Congr. Zool, 3, 59; 1963), but what is not well known is why clandestine matings should be frowned upon.

Buechner and Roth (Am. Zool., 14, 145; 1974) have investigated the possibility that the total population is divided into socially defined breeding demes, each of which occupies a lek. Most leks persist for long periods in the same place-of the 15 major leks serving the Torro Reserve now, 10 are in traditional locations which can be dated back at least to 1959. Evidence that animals breed only within a lek comes from tagging data and from translocation experiments. Most territorial males are found within 500 m of their home lek 90 to 100% of the time. Females are less attached to the site but all known individuals returned to the home lek for breeding. Until they reach 11 years when they settle down in a lek, fawns seem to have little attachment to any particular site but on no occasion did an adult male translocated to a foreign lek succeed in establishing itself.

Morphological data, such as ear, tail and hind leg length, provide further evidence of inbreeding. There was significant variation between leks in males' hind leg and ear length, and females' body and tail length. Yet more evidence came from the animals' altruistic behaviour which, according to Hamilton (Man and Beast: Comparative Social Behaviour, edit. by J. F. Eisenberg and W. S. Dillon, 59 Smithsonian Institute Press, Washington; 1971) is directly related to the degree of kinship within a population.

### Raindrops in the laboratory

from our Chemical Physics Correspondent

Drops of water, and eventually rain, grow from small droplets as they fall under gravity through slightly supersaturated air. Such a system is difficult to study in the laboratory as the droplets whose growth is of interest do not remain long in the field of view of any microscope and, unlike snowflakes, the drops can not reasonably be collected and studied at leisure.

Gollub, Chabay and Flygare (J. chem. Phys., 61, 2139; 1974) have applied the special properties of the argon ion laser to this problem with great success. Basically the droplets scatter the laser light and, because of their falling motion, they impart a Dopper shift to the light frequency of the order of 500 Hz. The exact shift will depend on the velocity of fall of the droplet through the viscous air and hence on the size of the droplet which can therefore be deduced from the Doppler shift. The shift can be accurately measured by beating the scattered light with light, unshifted in frequency, scattered from the stationary walls. The effective scattering volume is only 0.02 cm3 and is well defined in location so that the droplet size can be measured as a function of position in the sample chamber. This is 3.5 cm high internally and 10 cm in diameter so that control of temperature, of air purity and of supersaturation are straightforward to apply. Clearly different conditions are possible but in the experiments reported at 7° C and a degree of supersaturation of the order of 4%, the mean droplet radius is about 3  $\mu$ m towards the top of the chamber and increases to about 7  $\mu$ m as the particle falls to the bottom. At any specific point the distribution of drop sizes about the mean for that point is remarkably narrow, for example,  $\pm$  0.2  $\mu$ m in 4  $\mu$ m about 2 cm below the point at which nucleation occurs.

Confirmatory evidence on the particle size measurement is obtained from the scattering intensity. The growth of the droplets as a function of the distance they have fallen provides information about the growth mechanism; the results support a reasonably high thermal accommodation coefficient and even the value unity is not excluded. The condensation coefficient seems to be less than unity but doubtless further work will improve the details of the theory of drop growth. Though the results from these first experiments are important, probably even more important is the introduction of a new, informative and fairly simple and inexpensive technique which will enable the formation of water droplets and of rain to be extensively studied in the future.

The kob antelopes show altruistic behaviour in that individual males do not pursue females into neighbours' territories, victors of border disputes do not inflict serious wounds on losers, and individuals expose themselves in a conspicuous manner to predators inviting attack from a 'safe' distance. The latter behaviour has been investigated in detail by Smythe (Am. Nat., 104, 491; 1974).

Thus the courtship customs and social behaviour of each lek seem effectively to prevent matings outside the lek. Such matings are rare and their illegitimate offspring rarely survive. Social behaviour therefore maintains the genetic heterogeneity of the population by preserving the differences between leks. This ensures that there is sufficient genetic scope for evolution if conditions change, a mechanism which is probably especially important for the preservation of alleles with a small selective advantage (Wright in The Evolution of Life, edit. by S. Tax, 429, University of Chicago Press; 1960). A solution also exists in this successful species population to the problem of maintaining a certain amount of gene flow-every so often temporary leks are formed, lasting one to five years, after which time animals return to their original lek.

### Phonon transmission

from P. V. E. McClintock

THE transmission coefficient of phonons travelling from a silicon crystal into superfluid helium exhibits a maximum when the phonon energy corresponds to a temperature of about 5 K, according to a thermal pulse experiment by Swanenburg and Wolter of the Philips Research Laboratories at Eindhoven described in a recent issue of *Physical Review Letters* (33, 882; 1974).

The thermal energy in a crystal at low temperatures takes the form of quantised lattice vibrations known as phonons, which behave much like a gas of free particles each travelling at the velocity of sound. At any given temperature T the average energy of the phonons present is  $\sim kT$  where k is Boltzmann's constant, so that an increase of T not only causes an increase in the phonon density, but also brings about an increase of their average energy.

It has long been known that, when heat passes from one material to another, there is a discontinuity in temperature at the junction. The phenomenon can be understood in terms of an acoustic mismatch theory: just as in the case of light which undergoes partial reflection when incident on the interface between two media in which its velocity is different, so also are

phonons partially reflected if the velocity of sound differs in the two materials, thus affecting the transmission of thermal energy between them.

The velocity of sound in liquid helium is about an order of magnitude smaller than in most solids, so that there is a correspondingly large temperature discontinuity when heat passes between liquid helium and a solid, describable in terms of a thermal boundary resistance called Kapitza, the Russian physicist who discovered the effect. For  $T \le 1$  K values of the Kapitza resistance measured in equilibrium heat flow experiments are in reasonable agreement with those predicted by an acoustic mismatch model. When  $1 < \mathbb{Z} < 2$  K, however, the Kapitza resistance falls below the predicted values by an order of magnitude or more, an effect which is still not understood and which Swanenburg and Wolter have sought to investigate by means of their heat pulse technique.

Their experimental arrangement consisted of a very high quality silicon crystal, one face of which was in contact with liquid helium and the opposite face of which was in vacuum. A thin resistive metal film heater was deposited on the latter face. By applying an electrical pulse to the heater, high energy phonons could be generated, all of which necessarily passed into the silicon. A certain proportion of the phonons incident on the siliconhelium interface travelled on into the helium where they generated a pulse of the thermal wave mode known as second sound, which could be detected by a bolometer positioned a few millimetres away. The size of the bolometer signal was proportional to the energy flux entering the liquid. Because of the thermal discontinuity between them it was possible to heat the metal film to a very much higher temperature than that of the crystal. Thus it was possible to transmit phonons of energies equivalent to as much as 34 K into the crystal at 1.8 K, and then to investigate what proportion of these high energy phonons was transmitted into the liquid helium, which also remained at 1.8 K.

Unexpectedly, the authors found that although the transmission coefficient increased as the average phonon energy rose from 2 to 5 K, it then passed through a maximum and subsequently decreased as the energy was raised further. They describe a number of detailed checks, involving the use of heaters with different surface areas, which they have carried out in order to ensure that the change in the bolometer signal was really due to changes in the phonon transmission coefficient at the interface, and was not an artefact arising from some frequency dependent process in the crystal: they conclude that the phenomenon is genuine.

A new theory of he Kapitza resistance is therefore required which can not only explain why the transmission of thermal energy across a solid-liquid helium interface is anomalously enhanced above 1 K, but which is also able to account for 3 wanenburg and Wolter's conclusion that the responsible mechanism decreases in efficiency as T is raised above 5 K.

# High speed colour schlieren photography

from J. Kim Vandiver

THE cover picture of the bullet and candle is a colour schliesen photograph. It exploits techniques that were first used by August Toepler in 1864, and later by Schardin, who developed the first colour system in 1934. Though many improvements were made, until 1971 all colour methods gave only onedimensional information about the density gradients in the test section. In 1971 a technique was described (Strong and Settles, Scient. Am., 225; May 1971) which simplified the equipment and made possible colour photographs with fully two-dimensional density gradient information. These optical refinements were combined with a submicrosecond light source and triggering device to photograph a variety of transient events.

Refraction occurs when light encounters a region of non-uniform index of refraction. The refraction will be toward the region of increasing index in proportion to the gradient of the index perpendicular to the direction of travel. Natural events that cause changes in the pressure, density or temperature in the media around them ultimately alter the refractive index. Schlieren techniques discriminate light that has been refracted by a physical event from the remainder of the light in the test section. Once isolated, this light is focused by the camera into an image of the original disturbance.

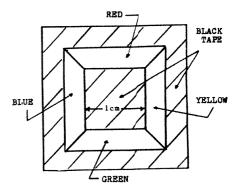


Fig. 1 Source filter

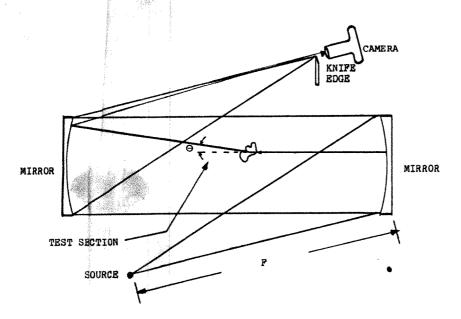


Fig. 2 Mirror system

Figure 2 depicts a simple schlieren system, consisting of two parabolic mirrors with a small light source at one focus and a knife edge and camera at the other. The test section is located in the collimated beam. The knife edge blocks the undisturbed image of the source, but lets pass light which has been refracted and deflected away from the knife edge. This yields a one-dimensional black and white photograph of the gradients in the test section.

The technique used here requires a multicoloured filter at the source, constructed as shown in Fig. 1, and four knife edges arranged to form a square aperture. The colour photograph so obtained indicates the direction of the gradient of the index of refraction by the hue of the colour in the photograph. The complete colour spectrum is used to provide 360 degrees of directional information.

Schlieren systems are very inefficient users of light. Most of the light available in the test section is rejected at the knife edges. To achieve properly exposed colour photographs requires intense light sources or long exposure times. To capture a supersonic bullet, or the shock waves from an electric spark, requires exposures of less than a microsecond. Our photographs employed an E. G. & G. Microflash Unit. This device discharges a 0.05 mF capacitor at 17 KV through a 2.5 cm air gap. Seven watt-seconds are discharged in  $3 \times 10^{-7}$  seconds. The light produced is focused by a reflector on to the rear of the coloured filter. Initial tests indicated that a small camera format and a fast colour film were required. A 35 mm format and High Speed Ektachrome (ASA 160) film were selected. A 400 mm telephoto lens was required to match with the 0.273 m

diameter, 2.46 m focal length parabolic mirrors, to yield an image size appropriate to the 35 mm format.

A variety of supersonic bullet photographs were taken. Bullet shock wave interaction with candle flames, soap bubbles, and solid objects was photographed revealing reflected, refracted and transmitted shock phenomena with excellent clarity. Bullet photographs were triggered, using a microphone, by the passage of the shock wave. Open air gap sparks, generating spherical shock waves, were photographed in air and water. Triggering was accomplished by a photocell and a variable time delay. Vortices are shed by propellor tips of an ordinary fan. These can be photographed when hot air from a flame is entrained by the vortex. Real time viewing with a synchronous strobe behind the filter is most revealing. More photographs may be found in Strong and Vandiver, Scient. Am., 231; 105; 1974.

The simplicity and versatility of this schlieren system are assets which make colour schlieren photography an attractive tool for scientific investigation, apart from its extensive use in supersonic wind tunnels. Other features include high resolution and sensitivity and pleasing colour results.

# Diseases of fodder crops

from A. I. H. Carr

FORAGE and fodder crops occupy about 75% of the agricultural area of the United Kingdom and contribute two thirds of the livestock intake. Perhaps surprisingly, the limitations imposed by disease on optimum output from these crops are only now becoming gener-

ally recognised. This was the opening theme of a symposium, "Diseases of forage and fodder crops" held at the British Museum (Natural History) on November 1 under the auspices of the Federation of British Plant Pathologists.

Introducing the topic, Dr A. J. H. Carr (Welsh Plant Breeding Station, Aberystwyth) referred to the difficulties of assessing yield losses in herbage crops which consisted of mixtures of outbreeding species grown together in competition, managed and utilised in many different ways, and with an ultimate yield assessed as animal production. Despite these difficulties, experience has revealed that infection with pathogenic fungi and viruses could cause adverse changes in botanical composition, loss in (and altered seasonal patterns of) productivity, and reduced nutritive value.

Dr A. J. Heard (Grassland Research Institute, Hurley) and E. T. Roberts (Agricultural Development Advisory Service, Reading) said that two thirds of grass crops surveyed had suffered damage due to disease and management problems in roughly equal proportions. They and others, referred to the damage to ryegrass, the predominant sown grass species, caused by crown rust, drechslera leaf spots and foot rots, and viruses such as ryegrass mosaic and barley yellow dwarf. Crown rust disease could reduce ryegrass productivity by 20% when compared with resistant varieties or plots protected by systemic fungicides and it also affected regrowth (Dr C. O'Rourke, Oak Park Research Centre, Carlow, Eire). The carbohydrate content of grasses could be reduced by half following infection with fungal leaf pathogens, and ryegrass mosaic virus reduced yield by 30% and accentuated winterkill. A valuable contribution came from F. G. Cook (Huddersfield Polytechnic) who described methods of relating production loss under various management systems to lesion development in ryegrass infected with Drechslera spp., which would have application to the study of other grass leaf pathogens. Dr E. G. Gray\*and J. F. Copeman (North of Scotland College of Agriculture) discussed the special problems arising where winter damage following extended snow cover is intensified by snow moulds (Fusarium nivale and Typhula incarnata). This influences the choice of ryegrass varieties towards those less susceptible to these pathogens. A different type of winterkill not associated with snow cover but dependent upon cold weather was described by Dr J. I. Holmes and Dr A. G. Channon (West of Scotland College of Agriculture). They showed experimentally that damage by Fusarium nivale was considerably increased if the inoculated grass was exposed intermittently to low temperatures.

Dr G. R. Dixon (National Institute of Agricultural Botany, Cambridge) drew attention to the increasing prolem of clover rot in white clover and discussed keys for assessing varietal resistance which existed in both red and white clovers.

The epidemiology of virus diseases, which are systemic and often transmitted both by contact and animal vectors, was the subject of papers by Drs R. W. Gibson and R. T. Plumb (Rothamsted Experimental Station, Harpenden) and by J. A'Brook (Welsh Plant Breeding Station, Aberystwyth). The Rothamsted workers, by using a filtered-air ventilated polythene tent over the crop to exclude mite vectors. were able to show that mechanical transmission played only a minor part in the spread of ryegrass mosaic virus. By contrast, the Aberystwyth work indicated that mechanical transmission was the predominant feature in the spread of the beetle-transmitted cocksfoot mottle virus. Large catches in the autumn in west Wales of aphids carrying barley yellow dwarf virus to grass and cereal crops were associated with low wind speeds, low precipitation and a prevailing southerly wind. Dr Plumb also drew attention to a number of virus problems in continental Europe which were potentially dangerous for the United Kingdom.

As the opening speaker had remarked, the solution to the problem of crop losses lay in applying the knowledge gained to the development of sound management techniques and the production of resistant varieties. Progress in resistance breeding was reported by P. W. Wilkins for crown rust and ryegrass mosaic virus and by T. D. Johnston for mildew and clubroot in swedes and rape (Welsh Plant Breeding Station, Aberystwyth). In view of past experience with pathogens of other crops it was gratifying to note that plant breeders were aware of the dangers of using simply inherited resistance, particularly to air borne pathogens, although for clubroot, caused by a soil-borne pathogen with a slower dissemination of genetic variants, such resistance could be beneficial.

Due emphasis was also laid on the nature of resistance. Mrs J. A. Chamberlain (Welsh Plant Breeding Station, Aberystwyth) presented evidence to show that tolerance in breeders' material to ryegrass mosaic virus was associated with certain ultrastructural features such as the size and number of pinwheel inclusions in host cells. Similar phenomena were associated with the tolerance of wheat to agropyron mosaic virus. J. A. Hargreaves and Dr J. W. Mansfield (University of Stirling) demonstrated the importance

of the individual components of the multiple phytoalexin response in controlling infection of broad beans by species of *Botrytis*.

Probably the most valuable lessons learnt at this symposium were the gains to be made in production from attention to management and disease problems, and the pitfalls involved in dealing with these complex biological systems.

# Herpesviruses and oncogenesis

from George Klein

The second meeting on this topic, held in Nürnbærg between October 14 and 16, dealt mainly with four types of virus known or suspected to be oncogenic in man or animals: Epstein-Barr virus (EBV) in man, Marek's disease herpesvirus (MDV) in chickens, herpesvirus saimiri and ateles in monkeys, and herpes simplex virus (HSV), defective mutants of which are thought to interact oncogenically with various animal or human target cells.

A central issue concerning EBV is the relationship of findings with animal experiments using virus-carrying cell lines in vitro to clinical findings in man. EBV is now widely accepted as the cause of infectious mononucleosis. Similarly, it may endow the lymphocytes with the capacity for continuous proliferation. Cells from such cultures, which carry multiple copies of the viral genome but only occasionally infectious virus, can produce grow malignantly in immunologically crippled animals such as nude mice.

#### Actiological alternatives

Some workers, including W. and G. (Children's Hospital, Phila-Henle delphia), Dr G. Miller (Yale University Schol of Medicine) and G. Klein (Karolinska Institute), believe that analogous virus-converted cell lines arise in mononucleosis patients or healthy seropositive individuals, but are held dormant, presumably by immunological means. Against this interpretation, however, A. B. Rickinson (University of Bristol) and his colleagues presented evidence from mixing the blood of acute mononucleosis patients with cord blood lymphocytes of the opposite sex. The emerging cell lines contain the sex chromosome of the added cord blood cells as frequently as that of the patient. Since continuous cell lines do not grow from uninfected cord blood cells, they infer that the cord blood cells were transformed by infectious virus released from some cell in the blood of the infected donor. Rickinson and his colleagues propose that EBV can remain latent in vivo in

a state like that of herpes simplex virus in the ganglia. The question remained unsettled, however, in the absence of data from mixture experiments that could have illumnated the derivation of lines from nomal seropositive donors.

In Burkitt's lymphona, EBV-genome tests on biopsy material by nucleic acid hybridisation or by EBNA (EBV-determined nuclear artigen) have led to an unexpectedly starp distinction between the endemic disease in Africa and the sporadic cases that occur elsewhere. Whereas 97% of the African lymphomas were found to carry the genome, the relatively snall number of tumours so far tested from elsewhere were uniformly genome legative. Other lymphoproliferative maignancies also proved genome negative irrespective of the serum antibody titre against EBV.

On the other hand, it has now been shown that cells from EBV-negative lymphomas in seropositive individuals can be superinfected with EBV in vitro. That suggests that those tumour cells which do carry the genome do so because they are derived from a genome-carrying clone, and not as a result of having picked up the virus in vivo. If this is accepted, the two main aetiological alternatives can be referred to as the 'immunological' and the 'cofactor' hypotheses. According to the first, the African BL patient differs from the normal EBV-positive individual only by some deficiency of his immune surveillance. (It must be remembered that BL is a relatively rare disease, even in the high endemic regions.) According to the cofactor hypothesis, EBV-carrying cells would not yet be fully malignant in normal individuals, and some other event would be required for full malignisation. Though at present it is impossible to distinguish between these alternatives, comparison of EBV-carrying lines derived from Burkitt lymphomas, if representative for the malignant clone, and EBV-carrying lymphoblastoid cell lines derived from normal individuals are pertinent to the question. J. E. Jarvis (University of Bristol) reported the presence of a chromosome 14 associated anomaly in 8 of 10 BL derived lines, apparently identical with the marker originally described by Manolov and Manolova. The marker was not found in other EBV-carrying lines. Together with the studies of Nilsson and Pontén that suggested a difference between the morphological, growth and marker characteristics of BL and non-BL derived lines, this evidence was at least consistent with the possibility that the BL lines may represent a special type of cell.

A remarkable change has occurred with regard to the second EBV-genome

carrying humai malignancy, nasopharyngeal carinoma (NPC). This tumour is notorous for its dense lymphocytic infiltration. Since only B lymphocytes were found to carry the viral genome in previous studies in vitro, it has been widely assumed that it is the lymphocytes that carry EBV in NPC. H. Zur Hausen's (University of Erlangen) in situ hybridisation data suggest, however, that the genome is associated with the epithelial cells. Nucleic acid hybridisation data on separated epithelial and lymphoid cells corroborated this conclusion. Klein, Giovanella and Lindahl showed, furthermore, that biopsy derived, nude mouse passaged NPC lines los the human lymphocytes but maintained the viral genome. The EBNA antigen was also present, clearly localised in the large carcinoma cells. These surprising findings will obviously reopen the whole question of the part played by EBV in the actiology of NPC. All available studies concur in suggesting that the genome is associated with the anaplastic or poorly differentiated NPC, but not with well differentiated squamous cell carcinomas at the same or other sites. This raises important questions about the susceptibility of the normal epithelial progenitor to EBV infection and about the part played by the virus in the genesis of the tumour.

In Burkitt's lymphoma, the striking time-space clustering of the disease in the high endemic areas gives clear evidence for environmental factors. In NPC, genetic factors may be more important. It is the most common tumour in certain ethnic groups of Chinese men but is very infrequent in Western populations. In Macao and in Thailand, intermediate incidences were reported for the offspring from intermarriages between the Chinese and non-Chinese populations. In Nürnberg, Simmons presented some new evidence suggesting that NPC patients differ from controls with regard to their HL-A constitution, in line with a postulated genetic risk.

#### Transformation in vitro

In the area of EBV-induced transformation in vitro, there is now firm evidence that different virus strains can differ in their biological characteristics. Y. Hinuma (Kumamoto University) reported differences in the growth, morphological and marker characteristics between lines transformed by the B95-8 and WIL strains. It was not clear whether the differences were determined by the resident viral genomes or reflected a slight difference in the affinity of the two virus strains for different types of lymphoid target cells. Independent comparisons by Menezes (University of Montreal), by Miller

(Yale University) and by Klein et al. showed differences between the P3HR-1 and the B95-8 virus strains. The strains both induce EBNA in comparable numbers of cells, but the unique P3HR-1 virus is unable to transform cord blood or BJAB cells, instead inducing an abortive viral cycle leading to the appearance of early antigen and to irreversible cell damage. In contrast, B95-8 virus has a regularly high transforming activity but fails to induce early antigen. This might suggest that the P3HR-1 line releases a non-transforming mutant virus, but E. Kieff (University of Chicago) reported that it has about 15% more DNA than B95-8 virus.

This has led to an interesting discussion between the herpes simplex workers, E. Kieff and B. Roizman (University of Chicago) in particular, and the EBV workers. Which is the wild type virus and which is defective? Looking at it from the lytic herpesvirus field, the transforming virus tends to be regarded as defective. But 'wild' EB virus recovered from the throat washings of IM patients has excellent transforming ability. It is clear, moreover, that the P3HR-1 virus does not induce a full lytic cycle in either the cord blood cell or the BJAB target, only short, abortive and suicidal stretches. It is also puzzling that the difference between the two virus strains is only apparent in relation to these target cells while in their own home strain both viruses seem to behave similarly. Both the B958 and the P3HR-1 cell lines have a proliferating, non-producer stemline, with occasional activation of the cycle in comparable, small numbers of cells. This is reminiscent of the lysogenic condition. Together with other evidence, for example differences in the producer status of foetal, newborn, adult and simian lines transformed by the same virus strain, this suggests that different lymphoid cells may vary in the degree of the restrictive control they can exert on a given virus strain. On the other hand, the P3HR-1-B958 virus comparison clearly shows that different virus strains may differ in the degree to which they obey the restrictions imposed by a given host cell. Somatic cell hybridisation experiments indicate that at least two different host cell controls are involved, one influencing the EA producing part and the other the late (viral DNA and viral capsid antigen producing) part of the cycle. Dominance of the more permissive character in the somatic hybrids suggests that both controls are of a positive nature.

The state of the viral genome in the transformed cells is of considerable interest. Nonoyama (University of Chicago) has reaffirmed his previous conclusion that at least a large part of

#### The Oklo phenomenon

from Peter J. Smith

In June 1972 a team of scientists from the French Atomic Energy Commission found that uranium samples from a mine at Oklo in the Republic of Gabon were depleted in uranium-235. In the first samples studied, the 235U content was only 0.003% down from the normal natural abundance average 0.720%; but more extensive work revealed that, in some samples, 224U accounted for only 0.40% of the total. Taking into account the total mass of the Oklo deposit, this meant that about 200 kg of ""U had apparently 'disappeared'-enough to produce about 10' megawatt-hours of ehergy in a nuclear power station and thus to power a moderate-size town for over 100 years.

The comparison with a power station is apt, for at a conference in Paris in September 1972 the French team proposed that the missing zisU had gone to fuel a natural reactor which had operated for a period of about 500,000 years some 1,800 million years ago. At that time, the 235U would have been more than four times as abundant as it is now. Neutrons produced by fission would be slowed down by environmental water, thus making them more effective in maintaining a chain reaction. Ultimately, however, the natural reactor would grind to a halt as the 235U burned up and as the surrounding water evaporated.

The reactor hypothesis is still unproven. But the issue may soon be resolved, for the French AEC has decided to release some samples of the Gabon uranium and its surrounding soil to the international scientific community. Some of the material has now been received by Dr S. A. Durrani of the University of Birmingham who plans to investigate the 'Oklo phenomenon' using thermoluminescence and fission track analysis techniques developed in connection with his study of lunar samples.

the viral genomes carried by non-producer cells exist in a free, plasmid-like form, although he could not exclude that some of the approximately 50 genomes could be covalently integrated with the host (Raji) cell genome. A. Adams Lindahl (Karolinska Institute) présented evidence for covalent integration, while she also confirmed that part of the genomes were free. The latter may be present in a circular form. Nonoyama also reported that he could reduce the multiple genome load of the Raji cell by cycloheximide

treatment. The state of the remaining genome has not yet been investigated.

While no restriction studies have been made on EBV as yet, Sugden (Karolinska Institute) reported the first EBV-transcription studies. He found a more extensive transcription of the viral genome (up to 30–50%) in the producer P3HR-1 line than in the non-producer Raji line, as expected. The viral RNA sequences detected in Raji cells were a subset of those found in P3RH-1 cells; they were sufficient to code for approximately 10 proteins. EBNA is the only known viral function in transformed non-producer lines.

**Immunological factors** 

Henle stressed the curious differences in antibody patterns between different EBV-associated diseases. Burkitt lymphoma patients with ongoing disease tend to form antibodies against one particular subcomponent of the early antigen complex (R). Mononucleosis and nasopharyngeal carcinoma patients preferentially make antibodies to another component (D). Since all known strains of EBV induce both antigens approximately simultaneously, during their cycle, these differences in the host response probably reflect the way in which the various antigens becomes available to the immune system. Considerable discussion followed the report of Henle that anti-EBNA antibodies appear very late, several months after the onset of infectious mononucleosis and are frequently low or absent in Hodgkin's disease (HD). Henle attributed this to the intranuclear nature of the antigen and postulated that considerable time is required before enough EBNA positive cells have been destroyed by the immune response. In HD the response would be often deficient.

The question why lymphoma, HD and other patients with EBV-genome negative tumours sometimes develop elevated EBV-antibody titres, approached by P. H. Levine and colleagues (National Institutes of Health). and by Johansson and colleagues (Karolinska Institute), respectively. Both dealt with the possibility that tumour-induced immunosuppression may be responsible, but in different ways. Levine found no evidence for any relationship between the lymphocytotoxicity reaction of Herberman against established lymphoma lines, and EBV titres. This reaction is mediated non-T lymphocytes, however. Johansson found a correlation between T-cell depression, as judged by PHA, Con A, and PPD lymphocyte stimulation or skin tests, and increased anti-EBV (VCA) titres. No such correlation was found with reactivity to a B cell mitogen (PWM). The notion that Tcell suppression may be crucial receives

support also from the demonstration by Aiuti that two congenitally T-cell deficient children had significantly elevated anti-EBV (VCA and EA) titres that fell to normal after thymus grafting and appearance of T cells in the circulation. It is likely that T cells play some regulatory part in maintaining the anti-EBV titre at a relatively low level from year to year in normal individuals. Depending on one's viewpoint, this could be attributed to T-cell mediated

#### Sabin withdraws claim

THE persistent irreproducibility and frustration to which Zur Hausen has alluded (see Klein on this page) is not confined to attempts to demonstrate herpesvirus DNA or antigens in cervical carcinomas. Albert Sabin has been obliged to withdraw his claim made early last year that a nonvirion antigen of HSV is associated with a large number of human cancers and can be found early in infection of guinea-pig cells in vitro (see Nature, 247, 334; 1974).

The history of frustration has extended over a period of five months during which Sabin has been trying to trace the reasons for his failure to reproduce the results he and Tarro reported last year (*Proc. natn. Acad. Sci. U.S.A.*, 70, 1032–1036 and 3225–3239; 1937). The irreproducibility of the results remains unexplained and Sabin's most recent paper (*Proc. natn. Acad. Sci. U.S.A.*, 71, 3248–3252, 1974) contains a detailed admission of defeat.

The difficulty in which Sabin now finds himself weakens the case for HSV as the aetiological agent of certain cancers; hopes of shedding light on oncogenesis through a study of the function of early HSV proteins are correspondingly dimmed.

surveillance against dormant, potentially neoplastic cells, or to some T-cell dependent antibody, acting either against dormant cells or against the spreading of periodically reactivated virus.

#### Marek's disease and herpes simplex

An important development in the field of Marek's disease is the establishment by Kato of three permanent lines which Nazerian has shown carry 70-80 genomes per cell. IUDR can activate antigen production in a small proportion of the cells. The lines had T-lymphocyte characteristics, confirming the suspicion that the disease is due to malignant T-cell proliferation. P. M. Biggs (Houghton Poultry Research Station) showed that bursectomy did not increase the incidence of the malignant lesion. Powell found that the

established lines can seve as targets for in vitro lymphocytotaicity tests. Preliminary evidence sugests that MDVinduced tumour cell may have a distinctive membrane antigen: clearly better antigen definition and immunological characterisation are urgently needed in the MD sysem. The disease seems to be uniquely sutable for studies on immune surveillante against tumours induced by a naurally occuring virus, with genetic differences in susceptibility and a corresponding contrast between self-limiting and malignant lesions. Biggs summarised the remarkable success story of vaccination against the malignant form, firs with attenuated MDV, and later with a partially crossreactive but non-pathogenic turkey herpesvirus (HTV). It is nteresting that without protecting birds from infection or reducing virus shedding the vaccine was nevertheless capable of preventing the development of malgnant disease. It is probable, although unproven, that this is due to immunisation against tumour-associated antigens. The situation may be analogous to the successful protection of hamsters noculated with polyoma or SV40 virus when newborn, by a second virus dose.

There is now increasing evidence that the HSV-transformed lines carry viral genomes. Frankel pointed out that viral RNA is often more easily detectable than viral DNA. Summers reported the detection of viral DNA using restriction fragments as the hybridisation probe.

Though laboratory models of HSV transformation have achieved a status of increased reproducibility, this cannot be said about the attempts to demonstrate herpesviral genomes or their antigenic footsteps in human cervical carcinomas. Zur Hausen said that this search has resulted in "persistent irreproducibility and persistent frustration, rather than persistent infection". This was countered by the argument that fractional genomes may be very difficult to demonstrate, as also evident from the in vitro transformed cell studies. The epidemiological evidence that links HSV type 2 infections to cervical carcinoma is circumstantial at best. While some careful studies suggest a relationship, at least in certain geographical areas and certain socioeconomic groups, the suspicion still prevails that the venereally transmitted type 2 infection may be another covariable of promiscuity, particularly the early onset of sexual life and multiple partners, factors that are known to have a role in the genesis of cervical carcinoma, rather than being an aetiological factor in itself. But recent epidemiological studies of the Melnick group where controls and cancer patients have been matched with regard to sexual history speak against this.

### review article

# Mutational pressure as the main cause of molecular evolution and polymorphism

Tomoko Ohta\*

The advent of detailed studies on the evolution of individual molecules has cast into doubt some of the neo-Darwinian concepts of what determines evolutionary change. Much more emphasis must now be placed on the constraints imposed by the structural and functional requirements of protein molecules, and on random fixation of very slightly deleterious as well as selectively neutral mutations.

As more data accumulate on evolutionary change at the molecular level, it becomes increasingly necessary to re-examine evolutionary theories<sup>1-4</sup>, and in particular the orthodox neo-Darwinian view that the rate and direction of evolution are determined almost exclusively by positive natural selection<sup>3-5</sup>. It is now clear that at the molecular level, evolutionary change results from the spreading of all 'tolerable' mutations in a population. But much of the theoretical argument depends on the definition of a tolerable mutation. Biochemists and non-population geneticists may equate the designation<sup>6-9</sup> with non-lethal, whereas to many population geneticists it means advantageous. There is, in fact, no clear distinction between tolerable and non-tolerable mutations: mutations occur continuously in any biological population, with a wide range of effects across the whole spectrum from lethal to advantageous.

Much recent evidence points to a continuum at one end of which are very nearly neutral mutations with undetectable phenotypic consequences, and at the other end of which are the mutations with a distinct phenotypic effect, and on which Darwinian selective pressures can therefore act. This point of view forms the basis for the present theory which can explain many of the recent data both on the rate of evolution as derived from studies of protein structure, and on the distribution of protein polymorphisms detectable as electrophoretic variants. Theoretically, it is possible to calculate the probability of fixation, or more generally the probability of mutants reaching a certain frequency within a population in terms of the "selection coefficient"10. If a mutant allele is selectively equivalent (neutral) to its wild-type allele, then the rate of mutant substitution in the population is equal to the mutation rate<sup>11</sup>. If the mutant is advantageous, the substitution rate is higher than the rate of mutational input and vice versa. Thus, tolerable mutations should include all those mutations which have a finite chance of survival, and the rate of evolution is determined by the average "selection coefficient" of new mutations 12,13.

This can be seen in the rate of evolution in various parts of the genome. It is now generally accepted that the less functionally rigid a molecule is the more rapidly it evolves<sup>5,6,9,14-16</sup>. For example, fibrinopeptides A and B and the middle segment of proinsulin are the fastest evolving molecules so far described and are enzymatically removed and discarded from the functional residue of the molecule<sup>5</sup>. The total genome DNA evolves at almost the same rate as these molecules<sup>17,18</sup>, which agrees with the proposal that the majority of genome DNA is not

informational in higher organisms<sup>15</sup>. This idea can be extended to the high variability of the "spacer" DNA of ribosomal genes19. Yet the evolutionary rate of fibrinopeptides may be slightly less than the mutation rate. This is because some structural constraint may affect polypeptide folding an so that the rate of evolutionary substitution of amino acids in fibrinopeptides is likely to be somewhat less than the intrinsic mutation rate. It is probable that molecular evolution proceeds essentially by mutational pressure rather than by positive Darwinian selection. More and more evidence is accumulating that the maximum observed evolutionary rate is close to the intrinsic mutation rates. If positive selection is the cause of such rapid evolutionary change, one must assume that, at any single period of evolutionary time, only a very few, specific, amino acid sites of a protein molecule can be replaced by definitely advantageous mutations. The remaining sites (constituting the majority) cannot accept mutations, since the fixation probability of definitely advantageous mutations can typically exceed 100 or 1,000 times that of neutral mutations in a large population. It is difficult to imagine that continuously changing environmental factors are so simple as to distinguish a few particular advantageous mutations among numerous others. Molecules are evolving individually, at a rate determined by the average selection coefficient of new mutations, which reflects the structural or functional constraints of each molecule. This view is essentially an extension of the neutral mutationrandom drift hypothesis of Kimura<sup>1</sup> and King and Jukes<sup>2</sup>, and takes into account the idea of "frozen accidents" proposed by Crick<sup>21</sup> and Ohno<sup>22</sup>. Once the structure and function of a molecule are determined in the course of evolution, natural selection acts mainly to maintain them, because all later evolutionary changes proceed under selective constraints. Natural selection then becomes mostly 'negative' and positive Darwinian selection is only a minor part of both total selection and the total number of mutant substitutions. If selection pressure is very weak, a mutant allele, even if slightly deleterious, can occasionally replace the original allele by random drift, particularly in small populations, and such chance events are likely to be important in molecular evolution 23, 24,

#### Higher order structures

Let us now discuss the effect of natural selection on secondary or tertiary structure, as natural selection acts through these higher order structures and not on primary structure. Probably the simplest example is the clover leaf structure of transfer RNA. I have suggested before that the coupled base substitutions in the paired region of this molecule, in the course

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of evolution, represent a very slightly deleterious base substitution in the first stage followed by a slightly advantageous complementary base substitution<sup>23</sup>. This hypothesis is supported by the observation of Holmquist *et al.*<sup>25</sup>. They have shown that G·U or U·G pairs account for about two-thirds of all non-Watson-Crick pairs in the helical regions of 43 sequenced tRNAs. They considered that since the presence of a single G·U pair does not interfere with helicity, it is an acceptable transitional stage. Other pairs such as A·C or G·A would cause greater distortion and therefore a mutation leading to such a change is less likely to spread in the population, and this non-random distribution of base pairs seems to fit the present hypothesis.

Transfer RNA is evolving at about the same rate as cytochrome c (ref. 25) and perhaps there is no fundamental difference between the evolutionary mechanisms of these two molecules. The molecular organisation of the secondary and tertiary structure of proteins is likely to be restored if another mutation is added after an amino acid is replaced in a well-organised system<sup>20</sup>. This is considered to be the basis of the observed linkage of amino acid substitutions.

This corresponds to Fitch's concomitantly variable codons (covarions)<sup>14</sup>. Wyckoff<sup>26</sup> by comparing bovine and rat RNases found that out of 18 changes which are involved in charge differences, about 14 are paired with respect to charge. Because of highly specialised intramolecular interactions<sup>27</sup>, the effect of the linkage of substitutions is apparently not the summation of the effect of each substitution. From these considerations, it is difficult to assess the adaptive significance, in ecological terms, of individual substitutions.

#### Selection pressure

Selection pressure must, in general, be very weak and indefinite for mutations that can only be detected at the molecular level, compared with mutations that have a visible effect. As neo-Darwinism is based on observations at the visible phenotypic level, it should be re-examined.

Consider a very small selective force as the lower limit of stronger selection. The majority of new visible mutations are deleterious, and the majority of "nearly neutral" mutations is likely to be very slightly deleterious, especially if the proteins and biochemical pathways are very highly organised and if environmental factors cannot interact directly with the primary structure of proteins. For such very slightly deleterious mutations, the chance of spreading by random drift is much higher in a small population than in a large population. In fact, if the product of the effective population number and the selection coefficient is less than unity, such mutations behave as if neutral and can spread through the population by random drift10. The product may be regarded as the number of effectively selected individuals per generation. This increases linearly with the increase of the total population size if the selective force is kept constant over a wide range whereas if the selective force is not constant, such a simple relationship may not hold<sup>28</sup>. As the product becomes larger, natural selection is more effective and very slightly deleterious mutations cannot spread23. Thus, the present hypothesis differs from the original neutral mutation-random drift hypothesis in emphasising a very small selection pressure. In fact, it is an extended form of neutral theory, conceived as the lower limit of the selective process.

Through extensive studies using *Drosophila melanogaster*, Mukai has shown that the mutation rate of viability polygenes is about 0.3 per genome per generation—much higher than previously thought<sup>29,30</sup>. This rate applies to those mutations which have effects strong enough to be detected by Mukai's viability test. If there is continuity of mutational change from disadvantageous to neutral, one would expect substantial numbers of nearly neutral mutations. Let us tentatively estimate the informational genome size of *Drosophila* from Mukai's

result. If the intrinsic mutation rate per base pir per generation is roughly  $10^{-8}$  as previously estimated<sup>5,15i1</sup>, then viability polygenes should correspond to  $3\times10^7$  bas pairs, and this amounts to one-third of the genome DM. Furthermore, if the total gene number in *Drosophila* is abot 6,000 (ref. 32), the average gene size becomes 5,000 base airs. Genes may include much of the regulatory DNA and the lass of mutations whose effects are not large enough to be deteted by Mukai's test. The latter class probably includes important candidates for "tolerable mutations". In my view, thee should be no distinct borderline between deleterious and neutral, between deleterious and advantageous, or even between deleterious and overdominant mutations. Random genetic drift and mutational pressure play a much bigger part than has been telieved.

#### Molecular polymorphisms

Mutational pressure is also important for the maintenance of molecular polymorphisms. Electrophoretically detectable alleles show a remarkably uniform distribution over a very wide geographical range in Drosophila33. This observation has been explained by the neutralists assuming migration34-36, whereas selectionists attribute it to some forms of "talancing selection"33,37. Very recently, by combining heat-denaturation studies with electrophoresis, Bernstein et al.38 lave found that each electrophoretic allele can be divided irto a few heat sensitive alleles at the xanthin dehydrogenase ocus in Drosophila. Their data, although not yet conclusive tecause of small sample sizes, suggest that these minor components within an electrophoretic class are geographically differentiated. They argue that electrophoretic alleles are maintained by balancing selection while heat-sensitive alleles may be selectively neutral. It is hard to imagine, however, particularly in the light of recent knowledge of protein structure and function27, that natural selection makes a clear-cut distinction between these two classes. It is more likely that genetic variabilities detected by electrophoresis in large populations like those of Drosophila represent a mutation-selection balance. For minor components (heat-sensitive alleles within each electrophoretic class), stochastic effects due to random drift are more prominent because of lower mutational input, and hence they may be geographically differentiated. Of course urgent investigation is needed to find out if the pattern observed by Bernstein et al. represents a general phenomenon.

So far, observations on protein variations in natural populations have been based mainly on protein electrophoresis<sup>39</sup> which only detects mutations which express themselves as charge differences on the molecule. To analyse such variations, therefore, we proposed a model of stepwise production of alleles in which the entire allelic states are expressed by integers, and mutations are represented by moving each allele one step either in the positive or in the negative direction on the allele space<sup>5,40</sup>. On the basis of this mutational scheme, we have investigated the theoretical consequences of weak negative selection (T. O. and M. Kimura, unpublished). In a typical example, we assume one type allele and multiple slightly deleterious mutations as follows:

mutation . . . 
$$v/2$$
  $v/2$   $v/2$   $v/2$   $v/2$  . . . allele . . .  $A_{-2} \rightleftharpoons A_{-1} \rightleftharpoons A_0 \rightleftharpoons A_1 \rightleftharpoons A_2$  . . . fitness . . .  $1-s$   $1-s$   $1$   $1-s$   $1-s$  . . .

where  $\nu$  is the mutation rate per gamete per generation and s is the selection coefficient assuming genetic selection. Under this model, it can be shown that the mutation-selection balance predominates if the product of effective population size  $(N_e)$  and mutation rate  $(\nu)$  is sufficiently large provided that  $s > \nu$ . The distribution is determined by the ratio  $s/\nu$  and the results conform quite well to the observed pattern of allelic distribution, that is, the commonest allele is surrounded by less common or rare alleles. Now, if population size becomes small so that  $N_e s$  is smaller than unity, very slightly deleterious alleles

become effectively neutral and the heterozygosity is determined by  $N_{ev}$  (ref. 41).

We have already suggested from a comparison of actual observations with the results of our simulation experiments assuming stepwis production of neutral alleles, that there are more rare allele than predicted from the strictly neutral hypothesis41. If we bring very slight negative selection into the model, the dscrepancy between the observation and the simulation should disappear completely. Perhaps the recent analysis of Yamizaki and Maruyama<sup>42</sup> needs re-examination in the light of ths. From data on protein polymorphisms, they found that the observed frequency of heterozygosity as a function of gene frequency follows a roughly uniform distribution in the range 0.0~0.5 (because of folding back the distribution around 0.5 (ref 43). They used data on several hundred electrophoretic allele and because the observed distribution agrees with Maruyama's theoretical prediction<sup>43</sup>, concluded that their analysis supports the selective neutrality of protein polymorphisms. If one uses the model of stepwise production of alleles by mutation, however, the distribution of the frequency of heterozygosty is not uniform unless  $N_e v$  is extremely small, even if alleles are neutral. Through an extensive Monte Carlo experiment assuming the step-allele model and selective neutrality, I have found that the frequency of heterozygosity gradually increases as the gene frequency class moves from 0.0 to 0.5 for the actually interesting range of  $N_{ev}$  (0.05 ~ 0.5). The observations on isozyme polymorphisms thus indicate once again the existence of more sporadic alleles than predicted by strict neutrality

#### Uniformity across species

A further argument against Darwinian selection as the sole determinant either of evolution or of polymorphism can be made on the basis of the remarkable uniformity of evolutionary rate and of heterozygosity between species. Individual cistrons seem to evolve at a very similar rate in diverse lines of organisms<sup>6,7,11,14,16</sup>. The existence of some local variations in evolutionary rate in closely related species12 does not materially affect the validity of this general statement<sup>3,5,44,45</sup>. If the majority of amino acid substitutions is due to positive Darwinian selection in response to environmental changes, one has to assume that such changes occur at roughly the same rate in various different lineages. It is hard to believe, for example, that the carp and the human lineages have experienced similar rates of environmental change. Similar arguments apply to the uniformity of the average heterozygosity per locus in different species. While this uniformity does not follow from the neutral mutations hypothesis, which predicts an indefinite increase in heterozygosity with increasing population size, it fulfils the expectations of selectionist hypothesis no better. Escherichia coli, for example, has an immense population size by comparison with Drosophila or man, and an entirely different ecology, but approximately the same degree of 'heterozygosity' in terms of effective number of alleles46

Approximate uniformity in the rate of evolution is to be expected if evolutionary change proceeds essentially by mutational pressure. The uniformity in heterozygosity between species can most plausibly be explained in terms of a balance between mutational and selective pressures. In a large population, heterozygosity is close to the maximum that can result from mutational pressure balanced against selection based on the structural and functional constraints of the molecules themselves. The constraints imposed by the structural and functional requirements of the peptides are taken as almost independent of ecological conditions<sup>20</sup>. The importance of such structural constraints is illustrated by the observation<sup>47</sup> that there is less heterozygosity for glycolytic enzymes than for other proteins, since the glycolytic enzymes might be expected to have the more rigid functional requirements<sup>48</sup>. If similar

distinctions are not seen in some mammalian species\*, this may be because they have not yet arrived at a mutation-selection equilibrium.

#### Enzyme differentiation and speciation

The patterns of enzyme differentiation between sibling species in the Drosophila willistoni group50 can most readily be explained by assuming that they have reached a mutation selection balance. The patterns of allelic variation in this group fall into two classes: (i) sibling species with the same set of alleles in similar frequencies; (ii) sibling species with non-overlapping allele distributions. For loci belonging to the first category, the same mutation-selection scheme applies to both sibling species, whereas for those in the second category, I suggest that the balance has shifted at the molecular level in the course of speciation. In a well adapted species, a constant input of very slightly deleterious mutations does not result in the replacement of the original allele if the population is very large. In small populations, which may often occur at the time of speciation, very slightly deleterious mutations behave as effectively neutral and may replace the original allele by chance. Then molecular constraints will be shifted, leading to a new mutation-selection balance. Thus, the degree of neutrality of new mutations is crucial to my theory of what determines the rate of evolutionary change and speciation. If the borderline cases between slightly deleterious and completely neutral mutations are important, as I assume here, then the population size becomes most crucial and the rate of evolution is higher in small populations such as may occur at the time of speciation. It is suggestive that the amino acid substitutions seem to be concentrated at the branch points in the phylogenetic trees of cytochrome c and the haemoglobins  $^{51-53}$ . This tendency, however, may be a statistical artefact resulting from the assumption of maximum parsimony which leads to the postulation of hidden mutational substitutions. The true pattern of molecular evolution and variation can only emerge from further investigation, bearing in mind particularly the importance of intramolecular organisation which derives from the polypeptide folding mechanism. It is this that determines molecular function and hence is subject to natural selection, and here ecological conditions are likely to be much less significant than has traditionally been supposed. In the light of such considerations, the original concept of the integrated gene pool<sup>54</sup> may have to be modified.

Finally, the distinction between the strict neutral theory and my theory should be clarified. (i) The neutral theory classifies new mutations into discrete classes; deleterious, neutral and advantageous classes. In my theory, there is no clear-cut distinction among these classes and the borderline cases are assumed to be more important. (ii) The rate of amino acid substitution is higher in small populations in my theory, whereas it is independent of population size in the strict neutral theory. Consequently, my theory predicts that substitutions will be concentrated at the time of speciation, when small population size creates a "bottle neck". (iii) In very large and stable populations, my theory gives an upper limit for heterozygosity.

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# articles

### Sequence analysis of immunoglobulin light chain messenger RNA

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RNA and DNA sequencing methods are used to define some features, principally at the 3' terminus, of the structure of immunoglobulin light chain mRNA. The results show that there is one molecule coding for the V and C regions of the light chain and rule out the possibility of two separate molecules.

A DETAILED knowledge of the structure of messenger RNA is required for a full understanding of its function, to define not only its coding properties but other less well known properties, such as control signalling and transport. For this, the sequence of those parts of the mRNA not coding for protein—the untranslated regions—is of special importance. Until recently, technical difficulties have confined such studies to bacteriophages. But a start has now been made on the structure of the eukaryotic mRNA coding for immunoglobulin<sup>1</sup> and globin<sup>2-5</sup>. The structure of the immunoglobulin mRNA is particularly important because of its relevance to basic problems of immunology. Light and heavy Ig chains contain a variable region (V region) which defines the antibody specificity and a region which is constant for each type and class of chain (C region). It is believed that the chains are coded by separate pools of V and C genes and that each chain is the result of the expression of a single gene from each pool<sup>6</sup>. To determine the number of genes involved in each pool by hybridisation techniques, a knowlege of the structure and purity of the mRNA is required. In addition the critical question of whether the integration is achieved at the DNA, RNA or protein level has been the subject of considerable speculation. A number of experiments indicated that the chains were synthesised from a single mRNA molecule rather than from two<sup>7-9</sup>. Objections, however, have been raised and direct structural evidence is essential to settle finally the argument.

In this paper we present further sequence studies on the light chain mRNA of the mouse myeloma, MOPC 21. We describe the sequence and location of the larger T<sub>1</sub> RNase oligonucleotides within the mRNA, and the sequence of 52 nucleotides adjacent to the poly (A.) These are included within a region of about 200 untranslated bases in the 3' terminal position. Overall the results establish the presence of a single molecule involved in the synthesis of both V and C regions.

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Characterisation and location of larger T<sub>1</sub> endproducts

Methods for puriving light chain mRNA have been developed by ourselves<sup>1,10</sup> and others<sup>11–13</sup> for a number of different mouse myelomas. The furity achieved is difficult to assess because of the different critria used by different workers. We have consistently used cells which have been grown in tissue culture because they repesent a much better starting material than the

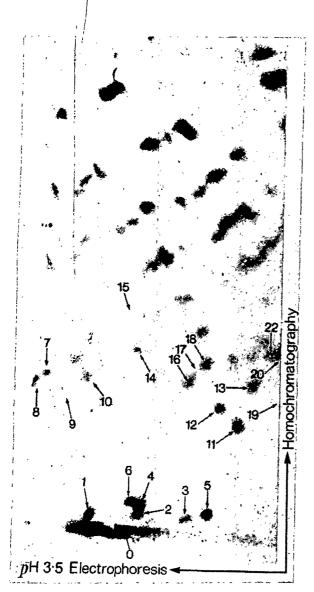


Fig. 1 Two-dimensional fractionation of a T<sub>1</sub>-RNase digest of <sup>32</sup>P-labelled light chain mRNA, purified from microsomes by successive sucrose gradient centrifugation and dT-cellulose chromatography¹ and finally by acrylamide gel electrophoresis in 8 M urea¹º. Fractionation in direction 1 is by ionophoresis at pH 3.5 in 7 M urea using cellulose acetate ('Cellogel'); direction 2 is by homochromatography on DEAE-cellulose thin layers (1:7) using a 3%, 10 min hydrolysed nucleic acid mixture. The numbered spots were eluted and analysed by further digestion with pancreatic RNase. We were unable to use these fingerprints for more detailed sequence studies as the radioactivity in the oligonucleotides was insufficient. To prepare oligonucleotides with enough radioactivity for further analysis, a less pure preparation had to be used in which the last step of purification was omitted. Fingerprints of the 30-50% pure mRNA were more complex but all the spots numbered in the figure could be clearly identified. They were eluted and analysed by further digestion with pancreatic RNase and separately by digestion with U<sub>2</sub>-RNase¹8. Spot 21 is not shown here but runs just to the right of, and midway between, spots¹19 and 20 (see Fig. 3). Spot 0 is unresolved from the streak at the origin (poly(A)) here. It was resolved in other experiments by using a stronger homomixture (5% unhydrolysed) in the second dimension.

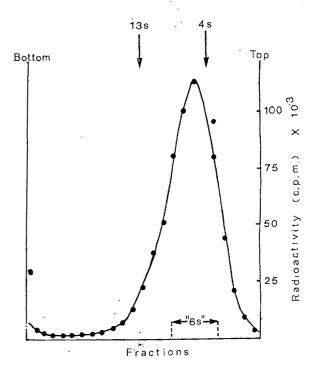


Fig. 2 Sucrose density gradient centrifugation of partially hydrolysed light chain mRNA. About 1 μCi of <sup>32</sup>P-labelled mRNA (30-50% pure material) was incubated for 25 min at 40° C in the presence of 50 mM Na<sub>2</sub>CO<sub>3</sub> and 40 μg yeast RNA. After addition of 3% SDS, 10 mM Tris Cl (pH 7.0) and 5 mM EDTA and incubation for 5 min at 70° C the fragmented RNA was fractionated in 15-30% sucrose density gradients in 100 mM NaCl, 5 mM EDTA, 10 mM Tris Cl (pH 7.6) and 0.5% SDS in the MSE 6 × 14 ml swinging rotor (35,000 r.p.m. for 17 h at 30° C). A peak of radioactivity (6S) was poled and fractionated by dT-cellulose chromatography. The poly(A)-containing fraction (20% of the total) was precipitated with ethanol and subjected to fingerprint analysis (see Fig. 3). 4S is the position of an internal unlabelled marker of E. coli tRNA and 13S that of intact (unhydrolysed) mRNA run in a parallel gradient.

more generally used solid tumours grown subcutaneously in mice. Furthermore, the tissue-cultured cells are ideal for the preparation of <sup>32</sup>P-labelled mRNA, which is then suitable for purity studies by chemical characterisation. Chemical methods are a necessary complement to purity determination based on less reliable procedures such as homogeneity on separation systems or specific translation in heterologous cell-free systems.

In a previous report<sup>1</sup> we described the preparation of purified light chain mRNA. Four oligonucleotides, ranging in size from 19-22 bases in length, were isolated from partially purified light chain mRNA and aligned with specific regions of the amino acid sequence of the protein. These four oligonucleotides-and others, described below—were prominent spots in T<sub>1</sub>-ribonuclease fingerprints, but were superimposed over an obvious background of spots presumed to derive from contaminating mRNA. We were then able to use these assigned oligonucleotides as chemical markers for the presence of light chain mRNA. Further purification was achieved by acrylamide gel electrophoresis where four bands were resolved. The light chain mRNA was identified as the only band containing the marker oligonucleotides in T<sub>1</sub>-ribonuclease digests. Evidence of purification was also provided by the much lower level of background oligonucleotides in the fingerprint.

Figure 1 is an example of a fingerprint of light chain mRNA purified by acrylamide gel electrophoresis showing the marker oligonucleotides (spots 2-5), with only a very faint background of contaminating material, and many other products in yields equimolar<sup>10</sup> to the markers, as would be expected from a pure molecule of the size of 13S. These additional oligonucleotides must, like the markers, derive from the mRNA and all those

	Table 1 Alignment or non-alignment of T1 end-products wi	Alignment or non-alignment of T <sub>1</sub> end-products with coding region of mRNA		
Product	· A.	Alignment†	Ligh chain (residue	
(Fig. 1)	Probable sequence or partial sequence*	(V, C or X)	nos f amino acids)	
0	$(A_3-C, A-C, C_{26}, U_{12})G$	X	•	
1§	$(A-U_2, C_9, U_5) A_2-G$	X (3)		
2§	U-A-U-C-C-A-U-C-U-U-C-C-C-A-C-C-A-U-C-C-A-G	C }	115–122	
3§	A-C-C-C-A-A-U-C-U-C-C-C-A-A-A-U-C-C-A-U-G	V 🏃 🐪	5 11	
4§	A-C-A-U-C-A-A-C-U-U-C-A-C-C-C-A-U-U-G	C ·	· 200–206	
1§ 2§ 3§ 4§ 5§	A-A-C-A-A-C-U-U-C-U-A-C-C-C-A-A-A-G	C .	137–143	
6	$(A_4-U, A_2-U, A-U, C_3, U_2) A_3-G$	X (3)		
7	$(A-U_2, C_2, U_4) G$	X (3)		
8‡	C-U-A-A-U-A-U-U-G	X (3)		
.9	A-U-U-U-C-A-C(U, C) U-G	V :	. 70– 73	
10	A-U-U-A-U-C-A-C-U-G	V ;	35– 88	
11a	$(C_{5-6}, U_2) A-C-A-A-G$	X (3)		
11b‡	A-A-A-U-A-A-A-C-G	Switch ;	,, , 05⊱108	
12a & b¶	$(A-C_3, C_2, 3, U_2) G$	X, X (3);		
13	A-C-A-U-A-A-C-A-G	C ;	. 88–191	
14	C-U-A-U-A-C-C-U-G	C i	. 91–194	
15	U-C-A-C-C-U-U-G	v :	19- 21	
16	A-C-A-U-C-A-A-U-G	C .	.13–146	
17	U-A-U-C-A-A-C-A-G	V	36– 38	
18	C-U-U-C-A-A-C-A-G 0	C · ·	08-211	
19	C-C- <sup>1</sup> A-C-U-C-A-C-A-A-G	C C	96–199	
20	C-A-C-C-U-C-A-C-G		77–180	
21	$(C_4, A-C_2) A-G$	X (3)		
22	C-A-C-Ç-U-A-C-A-G	<b>C</b> .	71–174	

<sup>\*</sup> Sequences were derived from results of both the pancreatic RNase and the U2-RNase end products of each spot1 and dignment with a predicted sequence derived from the known amino acid sequence. Where only partial sequences are shown, the products of ancreatic RNase digestion are listed. In these cases the quantitative values are only approximate. Two oligonucleotides (not marked on Fig. 1) both between 10 and 15 bases long, were not included in the table because of incomplete sequence information.

V, C and X show the alignment of the oligonucleotide with either the variable (V), constant (C) or neither (X) region of the mRNA. (3)

shows that the nucleotide was in the 3' untranslated region (see Fig. 3).

† The sequence of 8 and 11 b was deduced solely from the sequence analysis and independently of the protein sequence. § Previously characterised¹.

longer than nine residues, as well as some smaller ones, have now been studied (Table 1). Of these, 16 (including the four previously characterised) are assigned to the coding regionfive corresponding to the V region, 10 to the C region, and one (11 b) to the link or 'switch' between V and C. This sequence corresponds to residues 105-108, as follows:

105 Glu Ile Lys Arg (G)AA AUA AAA CG

These are residues that are generally considered to be in the V region, except for the arginine which could be described as the first of the C region. Nine oligonucleotides (Table 1) did not fit with any of the sequences predicted by the protein sequence and should therefore belong to the untranslated region. If so, they should be located either at the 5' or the 3' end of the molecule. To investigate this problem we introduced a small number of random breaks in the intact mRNA by means of mild alkaline digestion. Then, the fragments of a given size range were fractionated by oligo (dT)-cellulose chromatography (Fig. 2). In this way, the segments of mRNA which remained attached to the poly(A) (3' end of the mRNA) could be investigated by fingerprinting. The relative simplicity of this fingerprint (Fig. 3) showed that the fractionation was specific, and the presence of poly(A) that it derived from the 3' end. A further indication of the simplicity of the fingerprint was the separation observed in the region of the smaller oligonucleotides (top of the fingerprint). Thus spots 51 and 52 were resolved from the background, whereas in the intact mRNA they were unresolved from other components of related sequence. The numbered oligonucleotides (Fig. 3) were characterised by digestion with pancreatic ribonuclease. Almost all of the nucleotides assigned to the untranslated region (1, 6, 7, 8, 11a, 12a and 21, but not spot 0, and possibly one of the components of 12, see Table 1) were present in the fingerprint, but the oligonucleotides assigned to the coding region (for example 2, 3 and 5) were absent or present only in very low yields (see below). Thus all the main components are located between the coding region and the poly(A).

The above results suggest that there is a considerable number of bases in the 3' untranslated region. A simple addition of the lengths of the oligonucleotides 1, 6, 7, 8, 11a, 12i, 21, 51 and 52 gives a value of 106-108 residues. This excludes all the oligonucleotides shorter than hexanucleotides and therefore is obviously an underestimate. Thus in the sequence of 52 residues immediately adjacent to poly(A) (see below) 13 residues in digests of T1 RNase occur as small oligonucleotides. If we include these we account for 120 residues. That leaves the other small oligonucleotides of Fig. 3, which at a conservative estimate make the minimum total up to 150 bases in the 3' untranslated region.

An alternative method for estimating the length of the 3' untranslated region was based on the measurement of the molecular weight of the 6S preparation. This ranged evenly from  $0.7 \times 10^5$  to  $1.7 \times 10^5$ , as determined by acrylamide gel electrophoresis in formamide<sup>1</sup>. We calculated, therefore, that the 6S fraction is a mixture of fragments varying in size from 220 to 530 bases. In a population of molecules such as this the relative yields of oligonucleotides in a T<sub>1</sub> ribonuclease digest will be inversely proportional to their distance from the poly(A) at the 3' end. An examination of Fig. 3 shows that most of the spots in the coding region of the mRNA are completely absent, although three spots, 18, 19, and 4, are present in low yield. These correspond to residues 208-211, 196-199 and 200-206 respectively of the light chain. The largest fragments of the 6S fraction, therefore, include about 60-80 bases corresponding to the last 20-25 amino acids of the light chain. Subtracting this figure from the longest fragments in the preparation (530), we calculate that the 3' untranslated region is about 450 bases long, including the poly(A). Assuming that the poly(A) is still 200 bases long (in these longest fragments), the length of the 3' untranslated region is about 250 bases. This length measurement is critically dependent on the accuracy of the molecular weight determination and on our ability to recognise marker oligonucleotides. Spots present in less than 10% yield of the major spots on the fingerprint of Fig. 3 are probably not detected.

Two oligonucleotides are present as a mixture and may be distinguished by their different 3' ends after U2-RNase digestion. At least one is assigned with the 3' untranslated region.

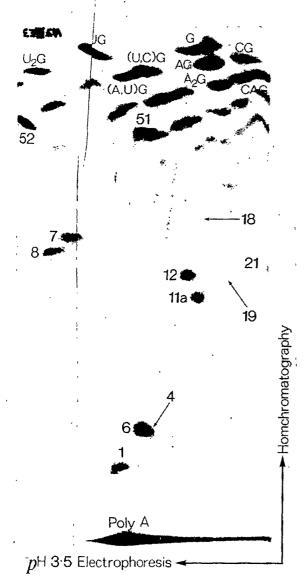


Fig. 3 Two-dimensional fractionation of a T<sub>1</sub>-RNase digest of the poly(A)-containing 6S fraction isolated by partial alkaline hydrolysis (Fig. 2). Fractionation was as in Fig. 1 except that a stronger 'homomixture' (5% unhydrolysed) was used for the second dimension. Spots were numbered from their position by comparison with Fig. 1, eluted and their identity confirmed by further digestion with pancreatic RNase. Spots 4, 18 and 19 were in too low yield for this check to be made. Spots 51 and 52 are relatively small oligonucleotides not isolated in intact mRNA. Their pancreatic products indicated that they were (A-C, U<sub>2</sub>, C) G and (U<sub>4</sub>, C) G respectively. The structure deduced for other smaller spots is shown directly.

This would give an overestimate of the length of the 3' untranslated region and 250 bases is likely to be a maximum value.

Despite the discrepancy in the values obtained (150 and 250 residues) we have argued that the lower value is an absolute minimum and the upper value probably a maximum value. Taking an average, we conclude that there are 200  $\pm$  50 bases present in this region.

Sequence of 52 bases adjacent to poly(A)

An alternative approach to sequencing the 3' end of the mRNA involves the use of the reverse transcriptase activity of DNA polymerase I from Escherichia coli<sup>2,5</sup>. With an oligo(dT)<sub>12</sub> as primer, synthesis of complementary <sup>32</sup>P-labelled DNA (cDNA) starts at the poly(A) or 3' end of the mRNA. Thus (<sup>32</sup>P) CTP-labelled cDNA was prepared using light chain mRNA

digested with endonuclease IV and fractionated (Fig. 4). The fingerprint obtained was highly characteristic (see legend to Fig. 4). The seven oligonucleotides numbered 1-7 (and two other spots—8 and 9—which were obtained from [a-32P] GTP-labelled cDNA) were sequenced using the techniques of partial exonuclease digestion, depurination and nearest neighbour analysis 15,16, to give the DNA sequence shown in Fig. 5. A comparison of this sequence with the results discussed in the previous section shows that it is complementary to some of the larger T<sub>1</sub> oligonucleotides shown to be in the 3' untranslated region of the mRNA (spots 6, 8, 51 and 52 of Table 1). Thus we conclude that DNA polymerase must be synthesising an accurate complementary sequence. The mRNA sequence may be drawn in the form of a double hairpin loop by maximising the extent of base pairing. The resultant structure is shown in Fig. 5a of the accompanying article5, where a comparison with a related region in rabbit β-globin mRNA is presented and discussed.



Fig. 4 Two-dimensional fingerprint of an endonuclease IV digest of (32P) dCTP transcript of immunoglobulin light chain mRNA. The mRNA preparation was contaminated with DNA. Therefore it was heated to 100° Cfor 5 min and digested with pancreatic deoxyribonuclease (0.2 mg ml-1) in 10 mM Tris chloride pH 7.5 and 10 mM MgCl<sub>2</sub> at 37° C for 30 min. EDTA was then added to 20 mM, the solution taken to 95° C and mRNA extracted using a phenol chloroform mixture (50:50) at pH 9.0. The RNA was concentrated by ethanol precipitation and transscribed using the Klenow subfragment of DNA polymerase I (E. coli) and Mn<sup>2+</sup> with [α- <sup>32</sup>P] dCTP (specific activity 100 Ci mmol<sup>-1</sup>,) and dATP, dGTP and TTP. The cDNA was isolated and then subjected to endonuclease IV digestion as described in the accompanying article<sup>5</sup>. Spots 5 and 6 were more prominent in other experiments. The unmarked prominent components near the origin of the second dimension are due to incomplete digestion by the endonuclease IV. In addition there is a complex background of weaker spots. Some are known to represent minor digestion products, while others are uncharacterised but may be due to longer cDNA copies or to transcripts of contaminating mRNA or DNA.

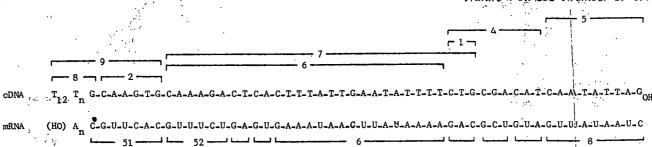


Fig. 5 Sequence of cDNA transcript of mRNA. The numbers above the sequence refer to the products of endonucless IV digestion shown in Fig. 4. The positions and sequences of the complementary T<sub>1</sub> oligonucleotides (Fig. 3 and Table 1) to this squence are as shown below the sequence. It should be noted that there is no formal overlap between the endonuclease IV spots 2 and 6 on the one hand and 4 and 5 on the other hand. The arrangement shown was established by the kinetics of labelling of the cINA.

Implications of the structural studies

A unique sequence of 52 bases has been established for the 3' end of the mRNA immediately adjacent to the poly(A). This is included within a region of 200 ( $\pm$  50) bases located between the coding region and the poly(A). In addition, a catalogue of 25 oligonucleotides ranging from eight to about 45 bases long is presented, and accounts for about 345 residues or one third of the length of the mRNA (excluding the poly(A)). Of these, 194 bases have been assigned to the coding region accounting for 75 amino acids out of a total of 214 of the light chain. From these results we can now propose a simple outline diagram (Fig. 6a) for the mRNA defining the approximate lengths of the various regions. Of particular interest are the 3' and 5' untranslated regions (3' and 5' UT in Fig. 6). The length of the 3' UT (200 bases) is probably accurate to within 50 bases, but that of the 5' UT region (150 bases) is much less accurate as it was derived by difference and is subject to the errors in all the other determinations1. A knowledge of the extent of the 3' UT region is critical to experiments in which complementary DNA is used for hybridisation with cellular DNA, designed to determine the number of V and C genes17. The function of such a long 3' untranslated region remains unknown.

Figure 6a shows the presence of V and C regions within a single mRNA molecule. An alternative model in which the V and C regions occur on separate molecules-illustrated in Fig. 6b—deserves special consideration in view of the existence of separate V and C genes. In both models we have used a length of 1,250 bases for all molecules because this is the size of the mRNA from which oligonucleotide markers for both regions have been isolated (see Table 1). Model b predicts that there should be two separate sequences for the two 3' UT

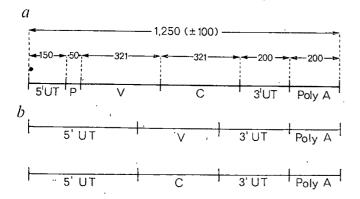


Fig. 6 Diagrammatic representation of the MOPC 21 light chain mRNA. a, Arrangement of the various regions and their length in numbers of bases. The lengths of the V (variable) and C (constant) regions are derived from a knowledge of the protein structure. The length of the 3' untranslated region (3' UT) is discussed in the text while that of the poly(A) and of the total mRNA is taken from ref. 1. P: precursor region<sup>19</sup>. b, Hypothetical alternative arrangement of V (variable) and C (constant) regions on two different mRNA molecules (see text).

regions. We have only found one such region the 52 residue long sequence shown in Fig. 5. Nevertheless, it ould be argued that the two mRNA molecules depicted in Fig. 6b have identical 3' untranslated sequences. If this wereso, the yield of oligonucleotides derived from the two regions hould be twice that of those nucleotides present in either the Vor the C region. The relative yields of some of the large T<sub>1</sub> olionucleotides of Table 1 have been measured<sup>10</sup> and are as follows: Spot 1 = 1.0; spot 2 = 1.3; spot 3 = 1.0; spot 4 = 1.1; spot 5 = 1.2; spot 6 = 0.8. It is clear that the yields of spot: 1 and 6, which derive from the 3' UT region, are equimolar witlin experimental error with those of various markers in the coding region (spots 2-5). Thus, the data contradicts the model. Inaddition, model b predicts that there are over twice as miny residues in untranslated regions of the mRNA as in the coding regions. Table 1 shows that 194 bases have been assigned to the coding region compared with 118 in the untranslated region. These figures again contradict model b, but lend support to model a. A 'switch' oligonucleotide spanning residues 105-108 of the amino acid sequence (spot 11b of Table 1) was isolated. Although this overlap of the V and C regions is rather too short to be considered as independent evidence for a single mRNA molecule it is fully consistent with it. Thus the structural studies reported in this paper confirm the hypothesis that there is a single mRNA molecule containing the sequence information for both the V and C regions of the light chain protein.

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### Sequence at the 3' end of globin mRNA shows homology with immunoglobulin light chain mRNA

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The sequence of 52 nucleotides adjacent to the poly(A) of rabbit \beta glolin mRNA has been determined. Striking sequence and structural homologies exist between it and mouse immunælobulin light chain mRNA. These homologies may be common to all mammalian mRNA.

THE messenger RNA (mRNA) coding for globin is an obvious choice for detailed sequence determination. It is easily purified and can be obtained in good yield1.2. All the mRNAs that have been purified including globin mRNA, are known to contain more nucleotides than are required for coding3. These extra residues, presumably present on each side of the coding region, and defined as the 5' and 3' untranslated regions, are of unknown function. They must, however, include control elements, presumably deined by nucleotide sequence. Functions such as translational control, the termination of transcription, the addition of poly(A), the binding of transport proteins and mRNA degradation are likely candidates.

Although globin mRNA has been available in a purified form for several years, the determination of its sequence has not been seriously attempted. RNA sequencing by radioactive techniques has been successfully applied to transfer, ribosomal and viral RNA'species, but has not been extensively used for mRNA because of its size and the difficulty of labelling it in vivo3. We report here the use of a new approach that overcomes these difficulties. Instead of labelling the mRNA strand in vivo, a radioactive DNA copy is synthesised by means of an RNA-dependent DNA polymerase. Reverse transcriptase, from various sources is well known to be active in this synthesis, but we have used, in preference, the reverse transcriptase activity of DNA polymerase I of E. coli which is very active in the presence of low concentrations of manganese4. An advantage in using a DNA polymerase is that synthesis starts only from a double stranded region. Such a region is defined at the extreme 3' end of the mRNA by hybridisation of oligo dT to the poly(A). Also, recent work in this laboratory<sup>6,8</sup> has established elegant procedures for the sequence analysis of radioactive DNA.

Globin mRNA sequence

Radioactive complementary DNA (cDNA) was prepared using each  $[\alpha^{-32}P]$ -labelled deoxynucleoside triphosphate of high specific activity in turn and the other three unlabelled triphosphates, with DNA polymerase and manganese. The conditions of incubation were identical to those previously described4 except that a subtilisin fragment of DNA polymerase I was used5, which lacks the 5' exonuclease activity of the enzyme. In these conditions short cDNA transcripts, about 50 nucleotides in length, were synthesised. The <sup>32</sup>P-cDNA was purified and digested with endonuclease IV (refs 6 and 7)

followed by two-dimensional fractionation (Fig. 1). The oligonucleotides thus separated were sequenced using the techniques of partial exonuclease digestion, depurination and nearest neighbour analysis6,8.

A detailed account of this work will be presented elsewhere, but as an example of the techniques that were used the fractionation of a partial venom exonuclease digest of spot 9 is shown in Fig. 2. The sequence of this oligonucleotide was tentatively deduced from the relative mobility of the spots8 giving the sequence as shown in the figure. Partial digestion with both venom and spleen exonucleases were particularly useful techniques. Once a successful digest and fractionation was obtained, as in Fig. 2, it was relatively straightforward to confirm the postulated sequence by using the standard methods of depurination and nearest neighbour analysis, with each of the four input labels. In this way, all the endonuclease IV products, except the very large ones close to the origin of the second dimension, were uniquely sequenced.

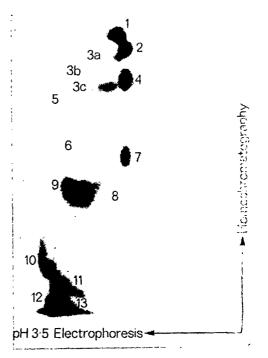


Fig. 1 Two-dimensional fingerprint<sup>22</sup> of an endonuclease IV digest of the cDNA of rabbit globin mRNA. [a-<sup>32</sup>P]-GTP-labelled cDNA<sup>4</sup> was purified by pancreatic digestion (10 µg ml<sup>-1</sup> pancreatic ribonuclease at 37° C, 30 min) followed by phenol extraction with a phenol-chloroform-isoamyl alcohol mixture (50:50:1) several times. The aqueous layer was ethanol precipitated, acid precipitated and dissolved in 30% triethylamine carbonate pH 10.0. Finally the triethylamine carbonate was removed by freeze drying with distilled water washes. The sequences of the spots are identified in Fig. 3.

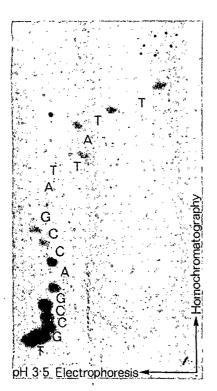


Fig. 2 Two-dimensional fingerprint<sup>22</sup> of a partial venom exonuclease digest<sup>6</sup> of spot 9. The sequence (5') - T-T-A-T-T-A-G-C-C-A-G-C-C-G-T (3') was deduced from the relative mobilities of the intermediates and confirmed in further experiments.

The final sequence was obtained by overlapping the sequenced oligonucleotides. This proved to be relatively easy, as amongst the oligonucleotides of Fig. 1 were larger products of partial digestion with endonuclease IV. Analysis of these products using depurination and nearest neighbour analysis gave the unambiguous sequences shown in Fig. 3. The overlap with the oligo  $dT_{12}$  was facilitated by a knowledge of the tetranucleotide sequence adjacent to the poly(A) in  $\alpha$  and  $\beta$  globin mRNA previously reported. The main overlapped sequence derives

from the higher yield oligonucleotides in F. 1. Some lower yield products, however, notably 5, 6 and 7 are unaccounted for in this sequence.

A problem in sequencing globin mRNA that, as usually isolated, it is a mixture of both a and B mNA, as may be shown by translation experiments in heterlogous cell free systems. From the sequence results described bove it seemed likely that our particular preparation of glbin mRNA, by contrast, contained predominantly one of th mRNA species (either  $\alpha$  or  $\beta$  globin mRNA) and relatively life of the other. To establish the identity of the major sequere, the mRNA preparation was assayed by translation in wheat germ extract. Characterisation of the 35S-methionine-ibelled product by the analysis of the labelled tryptic peptide showed that 5-10 times as much β globin as α globin was inthesised. We conclude that our mRNA preparation containspredominantly β globin mRNA and that this is the source of the major (52) sequence. Although the minor sequence of te a globin is incomplete, homology between the a oligonuclotides and the β mRNA sequence is apparent (Fig. 3).

#### Hairpin-loop structures

Figure 4 shows the  $\beta$  globin mRNA sequence complementary to the  $\beta$  globin cDNA) drawn in the form f two possible hairpin-loop structures, maximising the extent of base pairing. The stability of the hairpin loops may be assessed and the longer hairpin loop (labelled II in Fig. 4) has large negative free energy ( $\Delta G$  (25° C) = -10 kcalorie), suggesting that it is a stable entity. The smaller hairpin loop has ither zero free energy (Ia) or a value of +4 kcalorie (in Ib), suggesting that it is thermodynamically unstable.

In spite of this, we suggest that a double hairpin-loop structure exists and that it is stabilised by interactions with other parts of the mRNA or with specific protins. Of course, we admit that although plausible, such a secondary structure for the 3' end of globin mRNA may be totally incorrect, and that proof of this or any other arrangement must await the results of direct physicochemical studies. Whether or not the  $\alpha$  mRNA sequence has the type of secondary structure shown in Fig. 4 is also of great interest, but await further study. Considerable homology does exist, however, between the two mRNA sequences as would be predicted for two such functionally related molecules.

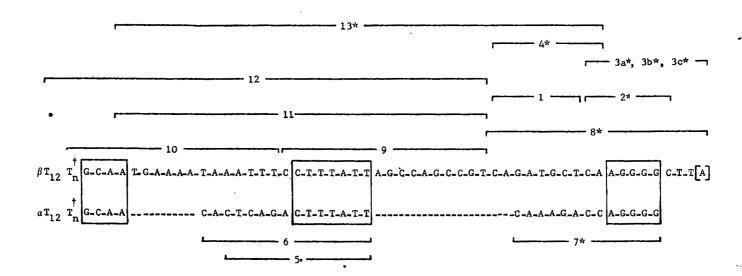


Fig. 3 Summary of sequence data on the oligonucleotides numbered in Fig. 1. The sequence for the cDNA of the  $\beta$  globin mRNA is fully overlapped. The oligonucleotides derived from the cDNA of  $\alpha$  globin mRNA are ordered by homology with the  $\beta$  sequence. Homology between the two sequences is denoted by the boxes.

<sup>\*</sup> The oligonucleotide's 3' end is a result of termination of transcription.  $\uparrow n$  varies depending on the position that the oligo  $dT_{12}$  primer hybrides to poly(A). The square brackets denote a sequence ambiguity.

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The amino ac sequence of rabbit β globin is known<sup>12</sup> so that, using the enetic code, it is possible to partially predict the mRNA sequice corresponding to the carboxyl terminus of the protein by the sequence reported here shows no homology with this reson and it is clear that it is entirely within the untranslated regin. A lower limit of 52 nucleotides for the length of the 3 untranslated region is therefore established. Our recent worl on longer cDNA copies of \( \beta \) globin mRNA has made it posible that the 3' untranslated region is substantially longerthan 52 residues. Molecular weight determinations of mous and rabbit B globin mRNA have suggested that  $\beta$  globi mRNA contains about 200 untranslated nucleotides13,1 (excluding the poly(A)), although it is not known how tese are distributed between the 5' and 3' untranslated regins. Moreover, it can be predicted from the studies on chin termination mutants15 of human a globin mRNA that a least 96 residues are present in the 3' untranslated region. Our results are consistent with these studies.

#### Globin and immunoglobulin mRNA sequences

The sequence of 52 nucleotides adjacent to poly(A) in the mRNA coding for the light chain of an immunoglobulin mRNA has been determined by similar methods to those described here and is described in an accompanying article. In this case, it was demonstrated that DNA polymerase in the presence of manganese, coies the mRNA faithfully. Thus, we have no reason to susject that the sequence reported here has any errors due to nistakes in transcription.

Figure 5 shows a comparison of the β globin and immunoglobulin light clain mRNA sequences. A striking sequence and structural homology exists between these two mRNAs, if the globin mRNA sequence is drawn in the 'b' form of Fig. 4. The homology between the two mRNAs may be considered in two parts, sequence and structure. Both possess the sequences A-A-U-A-A-G and U-U-G-C-poly (A) (enclosed within the boxed area of Fig. 5). These two sequences are also present

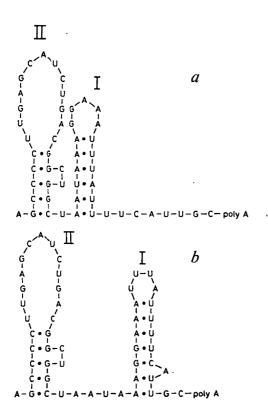


Fig. 4 Two possible conformations for the  $\beta$  globin mRNA sequence drawn in such a way as to maximise base pairing. I and II, hairpin loops whose estimated stability is discussed in the text.

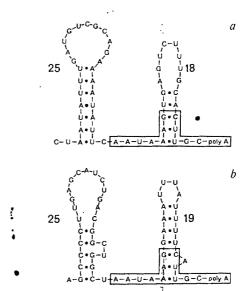


Fig. 5 Comparison of the 3' end of a, mouse immunoglobulin light chain mRNA with that of, b; rabbit  $\beta$  globin mRNA. Sequence in boxes denotes homology between two mRNAs. Number adjacent to each hairpin loop denotes the total number of nucleotides in that loop.

in  $\alpha$  globin mRNA (Fig. 3). A double hairpin-loop structure can be drawn for both  $\beta$  globin and immunoglobulin light chain mRNA (Fig. 5). The loops are identically positioned with respect to each other and are almost the same size in the two mRNAs. The sequence homologies mentioned above are in both cases at the base of the smaller loop and are again identically positioned. Much of the homologous sequence is therefore probably in an accessible unpaired region.

The striking similarity, both in sequence and in secondary structure between globin and immunoglobulin mRNA suggests that this untranslated region of the two molecules has a common function. One part of this sequence, that immediately adjacent to the poly(A) (U-U-G-C) may well be recognised by poly(A) polymerase<sup>17</sup>. This nuclear enzyme is thought to be responsible for the post-transcriptional addition of poly(A) to mRNA precursors<sup>18</sup> (HnRNA). *In vitro* studies have shown however, that this enzyme has no requirement for a specific sequence<sup>18</sup>. This apparent contradiction may result from different specificities *in vitro* and *in vivo*. Whether the rest of the structure is also involved in the recognition of poly(A) polymerase is more speculative, but it is tempting to suggest that the whole boxed area of Fig. 5 may be involved.

Messenger RNA is known to bind specific cytoplasmic proteins<sup>20,21</sup> which may be involved in general functions such as the transport of mRNA from the nucleus to the ribosome or control of the degradation of mRNA. The double hairpin-loop structure reported here could also be the recognition site for such proteins but such suggestions must be regarded as speculative in the absence of direct experimental evidence. Sequence studies on other purified mRNAs will reveal how general and therefore how functionally important are these sequence and structural homologies.

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### Early Tertiary hiatuses in the north-eastern Indian Ocean

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Cores from drill sites in the Indian Ocean have provided evidence of early Oligocene and early Eocene-Palaeocene hiatuses. That allows a determination of the depth of the carbonate compensation level during the Palaeocene. Possible reasons are given for the occurrence of the early Oligocene hiatus, which may be present at all depths and may well be a world wide phenomenon.

ONE of the major discoveries of the Deep Sea Drilling Project (DSDP) has been the frequent, oceanwide occurrence of discontinuities and hiatuses in the sediment record. Some of these are not just local phenomena but must be related to regional oceanic events1-2.

There are two types of hiatus: first, a hiatus in a continuously cored interval—this is indicated by missing palaeontological zones; second, a hiatus in an uncored or undateable interval. In the second case a hiatus is presumed to exist when the depth-age charts (with age bars showing the time range of each zone) unequivocally indicate that the sedimentation rate over a certain interval (the hiatus) is less than one tenth of the rates in the immediately overlying and underlying sediments.

There is a correlation between depth and age of the oceanic crust which holds in all oceans4. Subsidence curves based on this relationship, adjusted for the thickness of sediment between the ooze-clay boundary and volcanic basement<sup>5,6</sup>, can be used to determine the past depth of the carbonate compensation level, and also the depth range throughout which a hiatus occurred. Some hiatuses in the present sedimentary record are related to patterns of deep water flow7,8. Similarly, the ability to relate some of the older hiatuses to changes in deep and shallow water flow patterns will greatly increase the quantitative understanding of oceanic sedimentation.

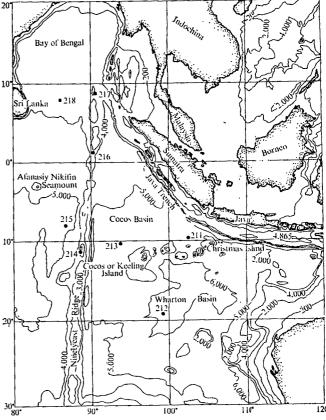
Results from Leg 22

The DSDP Leg 22 drill sites are shown in Fig. 1 and lithologies are summarised in Fig. 2. Cores from the Ninetyeast Ridge (Fig. 1, holes 214, 216, 217) showed calcareous oozes overlying a shallow water basal calcareous sequence. Volcanogenic material was also present at sites 214 and 216. The age of the basal sediment increases northwards. Holes 213 and 215, on

either side of Ninetyeast Ridge, show siliceou ooze passing down into brown clay and finally into calcarects ooze.

At drill sites 213, 214, 215, 216 and 217, hiauses centred on the early Oligocene can be inferred from the recovered cores, and at sites 214, 216 and 217 hiatuses can also beinferred in the early Eocene-Paleocene.

At site 211 two cores taken in a 100-m interval between



Leg 22 sites • plotted on the Russian bithymetric chart of the Indian Ocean (Udintsev, personal communication).

Pliocene and Matrichtian sediments contained small amounts of barren brownlay. At site 212 there was a total of almost 90 m of brown classeparated into several units by redeposited calcareous ooze, he clay must have accumulated over a period of about 80–10 Myr. Therefore, at both of these deep water sites hiatuses canot be inferred from our data (compare with the hiatuses repreted in Fig. 1 of ref. 3) because of the poor

fossil control and because the assumed sedimentation rate for brown clay was close to normal over the entire interval in question.

Cores from sites 213 and 215, on either side of Ninetyeast Ridge, imply a major hiatus in the mid Tertiary. At site 213 about 12 m of unfossiliferous clay separates the Middle Eocene from early Middle Miocene—a time span of about 30 Myr;

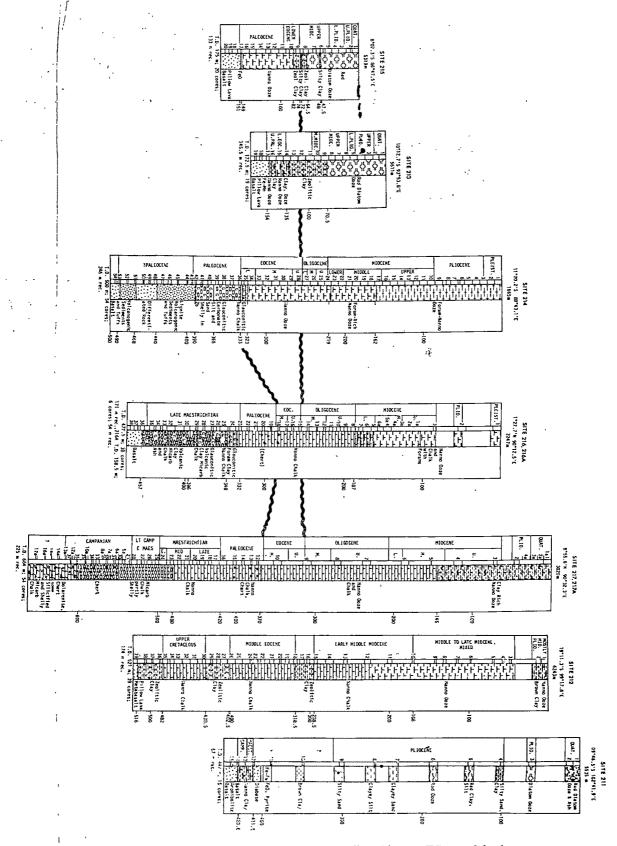


Fig 2 Stratigraphic logs of Leg 22 sites; wavy lines, hiatuses. T.D., total depth.

and at site 215 only 4 m of clay separates early Eocene from late Miocene—a time span of 40 Myr.

At site 214 two cores (20 m) span the entire early Oligocenelate Eocene interval—a period of 10 Myr—so it seems that a small hiatus can be inferred, because the accumulation rate is less than one tenth of the typical rate for pelagic calcareous ooze. At site 216 the nannofossil evidence indicates that the early Oligocene interval is only represented by about 2 m of sediment. One core from site 217 shows a hiatus from the late Eocene to the mid Oligocene.

In the core from site 214, between 336.5 and 334.5 m there is a hiatus of at least 4 Myr between Foraminifera zone P4 (Palaeocene-56 Myr) and zone P7 (early Eocene-52 Myr). At site 216 no early Eocene Foraminifera were recovered, although possible early Eocene nannoplankton were observed in the core catcher sample of one core. The upper portion of that core was Middle Eocene and the core immediately below was Palaeocene. Therefore, at best only a very small part of the early Eocene is preserved. At site 217 the early Eocene and topmost Palaeocene are represented by a very condensed sequence, unless part of the section is missing: Middle Eocene nannoplankton (Chiasmolithus grandis Zone) are separated from Palaeocene (Discoaster multiradiatus Zone) sediments by a 20m interval only.

#### Nature of hiatuses

The major hiatuses in the cores from sites 213 and 215, which are situated in the deep ocean on either side of the Ninetyeast Ridge, are centred close to 35 Myr BP, and the cores from sites 214, 216 and 217, on the Ridge, also revealed smaller hiatuses very close to 35 Myr BP. The cores from the latter sites also indicated small hiatuses between 50-56 Myr BP.

Using the palaeontological dates assigned to the major lithological units (Fig. 2) we plotted the sedimentary record of each site on to oceanic subsidence curves (Fig. 3). For the oceanic sites we computed the expected depth of the site from the empirical curve for normal oceanic crust4. We assumed that the sites on Ninetyeast Ridge were formed close to sea level, had a shallow water history, and subsided at the same rate as the Indian plate, to which they were attached10. We also adjusted the curves for sites 214 and 217 by eye, to give the existing oceanic depth (Fig. 3). From these subsidence curves we have assigned palaeodepths to various features of the sedimentary record.

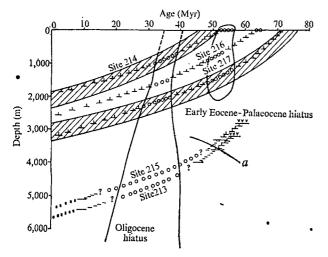
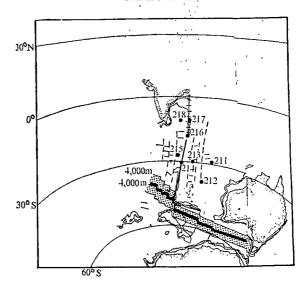


Fig. 3 Geological history of the sites, with hiatuses superimposed on oceanic subsidence curves. a, Carbonate compensation level; 000, hiatuses; careous sediments; SSS, siliceous ooze; ———, cl -, clay; basalt.



The region of the ocean floor postulated i be shallower than 4,000 m from the depth-age relationship superimposed upon the 32 Myr BP reconstruction of the relatie positions of Antarctica, Australia, and India 10. Assuming an oening time of 55 Myr BP the depth of the crust between Austraa and Antarctica would fall below 4,000 m for the first timespproximately 40 Myr BP.

Sites 213 and 215 record a change from carbnate to brown clay deposition at 3,600 and 3,300 m respectivey (Fig. 3). This effectively gives the approximate palaeodepth o the Palaeocene level of carbonate compensation in the eastern Indian Ocean.

The major hiatus at sites 213 and 215 occursit palaeodepths of 4,000-5,0000 m. Sites 214, 216 and 217 eacl show two hiatuses. The younger (Oligocene) occurs throughout depths between 1,000 and 2,500 m, and the older (earlyEocene) occurs. only at depths shallower than 2,000 m.

It therefore seems that the Oligocene liatus probably affected, to varying degrees, all of the site postions, irrespective of depth, and also that the Palaeocere-early Eocene hiatus was restricted to depths shallower than 1,500 m.

Evidence from DSDP legs in the Pacific and Atlantic indicates that the Oligocene hiatus is probably worldwide. We speculate that it may have developed in response to a worldwide readjustment of oceanic circulation. Such a realjustment could be a result of two major events which occurred during the Oligocene: during this Period there was interse glaciation in Antarctica<sup>11</sup>, and for the first time an effective deep water, circumpolar circulation system could have been established between Australia and Antarctica. Antarctica separated from Australia roughly 55 Myr BP (ref. 12). The subsidence curve data from the oceanic crust just north of Antarctica indicates that this region would have subsided from 2,700 n at the time of separation to more than 4,000 m by 40 Myr P. Such a depth would allow deep bottom water from further to the west to pass around the north of Antarctica, possibly establishing the Antarctic bottom water circumpolar current (Fig. 4).

We further suggest that the Palaeocene-early Eocene hiatus, which affects only shallow depths on the Ninetyeast Ridge, may be an event related solely to local tectonism.

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# letters to nature

### Optical variability of PKS0048-097

Hoskins et al. have identified the Parkes radio source 0048-097 with a neutral tellar object of 17 mag, and Carswell et al.2 have shown that, it common with the BL Lacertae class of quasistellar object BL Lacertids) it has a featureless optical spectrum, that it is a rapid radio variable at high frequencies3-6 and that it has a ralio spectrum similar to BL Lacertae<sup>3</sup>. (The name 'Lacertid' has been suggested' for this class, but this invites confusion withmeteor showers.) To complete the identification of PKS0048-017, we have investigated two further characteristics of the BL'Lacertids: the rapidity of the optical variability; and the total range of optical variability and its correlation with the decimetric pectral index8.

Thirty-three blue photographic plates from the Harvard archives, taker between 1921 and 1955 (27 between 1948 and 1955); were neasured with a Cuffey Iris Astrophotometer. Relative magnitudes were derived from a reference sequence around the object, which was obtained by a transfer from Mount Wilson Selected Area 117 (see ref. 8). The total amplitude,  $\Delta m_{pg}$  was found to be 2.7  $\pm$  0.3 mag. The available plates cover only a linited time and so this amplitude should be taken as a lower limit. In addition, on two occasions in 1948 the object exhibited rapid increases in brightness of about 1.4 mag in 55 d and about 0.9 mag in 30 d. Moreover, PKS0048-097 fits an empirical relationship, which exists for BL Lacertids<sup>8,9</sup>, between  $\Delta m_{pg}$  and the decimetric spectral index  $\alpha$ . From the measurements of flux density, S, of Shimmins et al.10 at frequencies, v, of 408 and 2,650 MHz, the value of  $\alpha$  (=  $d\log S/d\log v$ ) was found to be 0.33. This value of  $\alpha$  should only be considered as representative because of the known radio variability of the source3-6.

It is significant that a value of about 1.5 mag seems to be the lower limit on the total amplitude,  $\Delta m_{pg}$  for BL Lacertids with a substantial optical record9. This empirical result includes PKS0537-441, for which  $\Delta m_{yy}$  is at least 4.9 mag (ref. 11), as well as B20912+29 (ref. 12 and J. T. Pollock, unpublished). We conclude that radio sources with  $\Delta m_{pg} \gtrsim 1.5$  mag should be considered as prime candidates for the BL Lacertae class, and that the use of archival photometric records may be an efficient means of identifying new BL Lacertids.

Concerning the nature of PKS0048-097 and the BL Lacertids in general, Oke and Gunn<sup>13</sup> have shown that the extended object around the quasistellar source BL Lacertae has the spectrophotometric properties of an ordinary giant elliptical galaxy, with a redshift of 0.07. Similar results have been obtained by Wlerick et al.14. Furthermore, if we make the reasonable assumption that the K correction for this galaxy is about 0.1 mag, then it can be shown with the help of the Oke and Gunn data that the BL Lacertae galaxy fits the Hubble diagram of Sandage15 for radio and N galaxies within the scatter of the points. BL Lacertae is thus associated with an E galaxy that has an intrinsically high luminosity, but is otherwise a normal redshifted object, and it is reasonable to induce that all BL Lacertids have similar properties. The suggestion that these objects are blueshifted16 is thus unlikely to be

correct and therefore a major requirement of the local hypothesis for quasars (that they are ejecta from the Milky Way and/or nearby galaxies) apparently cannot be satisfied by the BL Lacertids. It follows that the problem still exists of accounting for the large power radiated by the BL Lacertids and other quasistellar objects at their cosmological distances.

To explain the energy requirements Hjellming<sup>17</sup> has suggested that quasistellar objects are singular 'white holes' which are multiply connected to black holes in our own or some other universe. According to Burbidge18, the basic concept of white holes originated in the writing of Sir James Jeans in 1929, and has occasionally been revived by Ambartsumian, Hoyle and others. Unfortunately, no theoretical predictions exist for comparisons with observations, and indeed, according to Hjellming (private communication) the hypothesis is highly speculative. Nevertheless, it is well known that most galaxies and many ellipticals have bright semistellar nuclei, and on the basis of morphology it would seem more reasonable to regard the phenomenon of quasistellar objects as an extreme case of bright galactic cores, with which are associated singularities caused by gravitational collapse, rather than white holes.

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#### Solar models with rotating cores

It is possible to achieve low solar neutrino fluxes with solar models with rapidly rotating cores<sup>1-3</sup>. But to achieve a flux consistent with the 1 SNU (1 SNU =  $10^{-36}$  neutrino capture per (target atom s-1) upper limit of Davis4, the rotation must be so rapid that the approximate treatment of rotation used is of doubtful validity. Further it is difficult to obtain a result simultaneously giving a flux less than 1 SNU and a solar oblateness consistent with observation<sup>5</sup>. Sakurai<sup>6</sup> has shown that it is possible for Eddington-Sweet circulation to transfer angular momentum both inwards and outwards in solar models with rotating cores on a time scale much less than the solar lifetime. Thus as the Sun evolves, its rotating core gets smaller while its outer part spins up. This suggestion has les us to investigate the possibility that this spin-up might lead to the onset of some transient phenomenon, such as the sudden beginning of thermal convection. Under special circumstances, a transient state can lead to temporarily depressed fleutrino fluxes7-10. It was hoped that the transient phase induced by spin-up, coupled with an already depressed flux due to the rotating core would lead to a flux of less than 1 SNU while requiring less extreme transients or rotation than is necessary when these effects are applied separately. In addition, a mechanism for the transient would be provided. Unfortunately this hope has not been realised and additional problems with rotating solar models have been discovered.

As in earlier work, we take rotation into account by multiplying the pressure gradient by a factor containing the average centrifugal force,

$$\frac{dP}{dM} = -\frac{GM(r)}{4\pi r^2}F, \quad F = 1 - \frac{2r^3\Omega^2}{3GM(r)}$$

The radiative gradient, as calculated from the luminosity and used in testing for thermal convection, must be divided by F. According to Sakurai's model, the radiative core of the Sun is initially rotating with a constant angular velocity. Because of induced circulation currents, angular momentum is transported from the surface towards the core. Additionally the outer regions can lose angular momentum to the solar wind. To maximise the final rotation rate, and because the angular momentum transferred to the solar wind depends on the surface rotation rate, we have neglected the surface loss of angular momentum. The Sun as a whole therefore conserves its angular momentum and as the surface layers rotate more slowly, the inner region must rotate more rapidly. The distribution of angular velocity in the transition region between the slowly rotating surface region and the uniformly rotating core region is governed by the condition that it must remain stable against shear flow turbulence. The onset of shear flow turbulence in a stratified medium occurs when the Richardson number

$$Ri = -g \mid (\partial \ln \rho / \partial r) - (\partial \ln \rho / \partial r)_{ad} \mid (\partial u / \partial r)^{-2}$$

is less than some critical value. In this formula g is the acceleration of gravity and  $\partial u/\partial r$  is the velocity gradient associated with the differential rotation. We recognise that other instabilities may be associated with the differential rotation, but feel that shear flow turbulence almost certainly will transport angular momentum efficiently even on a short time scale. Thus we have set an upper limit on the velocity gradient by requiring  $Ri \ge 1/4$ . The width of the rapidly rotating shell is then determined by the condition that this shell contains the angular momentum lost by the outer envelope. Since this shell contains not only its initial angular momentum, but also the angular momentum lost by the envelope, it must rotate more rapidly than the core. We should therefore apply the Richardson condition on both sides of the shell. But in order to maintain a relatively simple computing algorithm and test

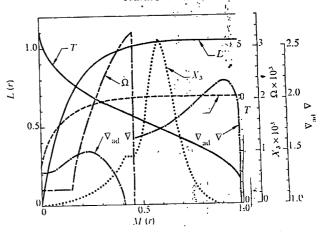


Fig. 1 Structure of sequence-B at an age of  $4.7 \times 1^9$  yr. Quantities shown are temperature T ( $10^6$  K), hydrogen has fraction  $X_3$  rotation rate  $\Omega$  (rad s<sup>-1</sup>), <sup>3</sup>He mass fraction  $X_3$ , ratio of adiabatic gradient to actual gradient in the star  $V_{\rm d}/V_{\rm t}$ , and the luminosity L ( $L_{\odot}$ ). The convective region is the small zone where  $V_{\rm ad}/V = 1$ . X = 0.69; F = 0.02;  $\Omega_0 = 3.10^{-4}$ ; Ri = 0.25;  $\varepsilon = 6.4 \times 10^{-3}$ ;  $\Sigma \Gamma \varphi = 13.2$ .

the importance of satisfying the Richardson condition, we have required the velocity gradient to obey his condition only on the inner side of the shell. On the over side of the shell we allowed the rotation rate to jump disontinuously to zero. Our procedure then was to choose some inform initial rotation rate  $\Omega$ . At later times we assume if the angular momentum exterior to a mass  $M\Omega$  has been transferred to the rapidly rotating shell. We assume that  $M\Omega$  dereases linearly with time. Our procedure produces a high pear rotation rate and enhances the possibility of inducing a transent. Normally the models were mixed only in those regions whee the criterion for thermal convection was satisfied althoughit would have been more realistic to mix in the region of differential rotation also. The difference between these two cases is small.

The oblateness in each model was calculted following Goldreich and Schubert11. Their equation (14)was integrated from the centre outwards and surface inwards and integrations performed until the oblateness and its derivative were continuous at some matching point. We find oilateness more than an order of magnitude larger than that found by Demarque et al.1 (DMS). We have obtained similar results from programs written independently by each author and have identified the source of the discrepancy. In the nodels studied there is a discontinuity in  $\Omega$  at the edge of the rotating core. The equation to be solved contains a term  $d\Omega^2/dr$  and thus must satisfy a jump condition at  $r_{\Omega} = r(M_{\Omega})$ . This is easily accomplished by letting  $d\Omega^2/dr = -\Omega_c^2 \delta(r-r\Omega)$  near the core edge, where  $\Omega_{c}$  is the angular velocity at the core edge and  $\delta(r-r_{\Omega})$  is the Dirac delta function. In our sequence B described below, which is equivalent to DMS sequence C4, we find  $\epsilon = 2.8 \times 10^{-3}$  and they find  $\epsilon = 1.59 \times 10^{-4}.$  If we neglect the jump condition, we find  $\epsilon = 1.58 \times 10^{-4}$ . Thus it seems that DMS have not properly dealt with the core boundary. We note that the large  $\epsilon$  would remain if  $\Omega$  at the core boundary dropped rapidly, but not discontinuously, to zero. As a further check, we made a crude approximation of the model shown in Goldreich and Schubert's Fig. 2 and find an  $\epsilon$  consistent with theirs.

We have computed two sequences (designated A and B) which are essentially the same as the DMS sequences C2 and C4. The rotating core was handled in the same manner as DMR rather than as described above except that mixing was allowed when the modified criterion for convection was satisfied. Much of the core was convective throughout the sequence. Some of our results for this case are shown in the first two columns of Table 1. The agreement with DMS is remarkable in most respects, even to the agreement of the fluxes from the individual reactions in sequence E. There are, how-

	Table 1 Pr	operties of the	11104013		
equence	Α ,	· B	C	D	E
nitial Ω/(rad s <sup>-1</sup> )	F = 0.50 =	const.	$3 \times 10^{-4}$	$3 \times 10^{-4}$	$3\times10^{-4}$
mitial X	0.718	0.712	0.69	0.69	0.69
initial $M\Omega/M\odot$	0.90	0.90	0.98	0.98	0.98
Final $M\Omega/M\odot$	0.07	0.146	0.456	0.456	0.456
Final M uniform rotation/Mo	0.07	0.146	0.146	0.133	0.073
Ri			0.25	0.25	0.50
Initial L/L <sub>O</sub>	0.19	0.046	0.99	0.99	0.99
Final L/Lo	1.11	1.00	1.05	1.04	1.06
Log Te		3.777	3.781	3,782	3.783
Σσφ/SNU '	0.50	0.79	13.2	11.3	9.3
Ι X <sub>c</sub>		0.66	0.248	0.248	0.242
$F_{\max}$	0.50	0.46	0.52	0.52	0.60
$\Omega_{\text{max}}/(\text{rad s}^{-1})$	-	$4.5 \times 10^{-3}$	$3.3 \times 10^{-3}$	$3.3 \times 10^{-3}$	$3.0 \times 10^{-3}$
Smallest radiative-convective inte	rface	0.0	0.422	0.398	0.421
	face —	0.161	0.467	0,467	0.450
ε×10+3	0.46	2.8	6.4	6.4	5.9
Program	RKU	RTR	RTR	RTR	RTR

ever, crucial deferences between our results and DMS. It is likely that the angular momentum lost from the core would have been transferred bymass-motion, possibly Eddington-Sweet circulation. Ulrich¹² has pinted out that this would play havoc with the light element abundances at the solar surface. Here we have not even made this assumption and mix only when instability for thermal convection exits. But during the very early stages, the convection zone is deep enough to enhance the surface ³He from 0 to  $1.5 \times 10^{-4}$  by mass. This is above the upper limit observed by Hall¹³. It will be exceedingly, difficult to produce rotating models which do not have such produms with ³He or the even more sensitive Li and Be.

As noted above, we find a much larger oblateness,  $\varepsilon = 4.6 \times 10^{-4}$  and  $2.8 \times 10^{-3}$  compared to their  $\varepsilon = 2 \times 10^{-5}$  and  $1.6 \times 10^{-4}$ . Our results are in strong disagreement with the observed value<sup>5</sup> of  $\varepsilon = 5 \times 10^{-5}$ , which can be taken at least as an upper limit.

There are several possibilities for inducing a transient phase with a core that is spinning up. First, we suppose that the outer part of the rotating core arrives at some part of the <sup>3</sup>He peak after 4.7×10° yr of evolution. This could well cause the simultaneous onset of thermal convection. Because of the stored energy in the <sup>3</sup>He and the modified radiative gradient inside the rotating core, it is conceivable that the convective region might grow. But our calculations show that mixing

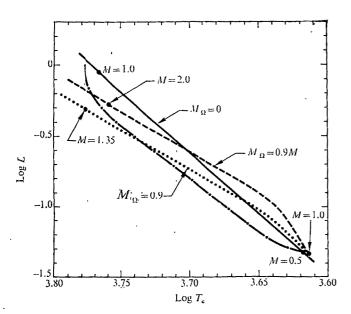


Fig. 2 H-R Diagram for main sequence models for the composition X=0.712. The solid line is the non-rotating main sequence, the long dashed line has  $M_{\Omega}=0.9M_{\odot}$ , the short dashed line has  $M_{\Omega}=0.9M$ ; in the rotating cores F=0.50. Reference marks  $(M_{\odot})^{\dagger}$  are shown on each sequence. The evolutionary track for model B is also shown (dot-dash line).

never occurs except for rotation rates almost as large as those used by DMS.

The results for one model (designated C) which developed a convective region are shown in the second column of Table 1. The initial rotation rate was  $3\times 10^{-4}\,\mathrm{s}^{-1}$ . While evolving for  $4.7\times 10^9$  yr the rotating core was reduced from  $0.98M_\odot$  to  $0.46M_\odot$ . At this point, a small convective region developed. Figure 1 shows the structure at this time. Comparison of our curve for  $\Omega$  and Sakurai's Fig. 2 emphasises the extremes we must have to get any effect. The convective region is on the inner slope of the <sup>3</sup>He peak. Further evolution with small time steps and no further reduction in the rotating core size show that the convective region does not grow, but rather shrinks in size. The curve for the ratio of the adiabatic gradient to actual gradient in the star,  $\Lambda_{\rm ad}/\Delta$  in Fig. 1, shows that the central core is extremely stable.

There are further problems with this model. To achieve the proper luminosity, X had to be reduced to 0.69 initially. Thus the neutrino flux ends up at 13.2 SNU which is more than double our standard Sun. Further, the oblateness of this model is more than 100 times larger than Dicke's value. Certainly any model which has any substantial rotational modification in the <sup>3</sup>He peak will suffer similar oblateness problems.

The results for a similar model in which the composition was mixed in the differentially rotating region is shown in column 4 of Table 1. It differs little from the previous model.

One additional possibility remains: if the differentially rotating region reaches into the central region of high nuclear burning, the addition of <sup>3</sup>He from the outer part of the model might lead to further mixing driven by nuclear burning. Such a sequence (designated E) is shown in column 5 of Table 1. Mixing has been allowed in the differentially rotating region and the inner edge of this region is at  $0.073M_{\odot}$ . The mixed region has been extended over a larger mass than sequence D by making the Richardson number larger. The region unstable to thermal convection extends from 0.421 to  $0.450M_{\odot}$  and again has no inclination to grow. The oblateness is still burge

So it seems that the only possibility for the spin-up to induce a neutrino-eliminating transient would be for the rate of spin-up to be extremely fast.  $M_{\Omega}$  would have to be reduced from say  $0.6M_{\odot}$  to  $0.1M_{\odot}$  in a time comparable to the <sup>3</sup>He lifetime at the centre ( $\sim 10^5$  yr). This rate of spin-up seems implausibly high.

The models proposed by DMS seem to be ruled out as solutions to the solar neutrino problem. (Roxburgh does not describe his models in sufficient detail for us to check, although they seem very similar to those reported by DSM.) Their oblateness is much greater than the observed value. Their <sup>3</sup>He at the surface is above the observed upper limit. Finally a problem which will afflict all such models is demonstrated

in Fig. 2. A non-rotating main sequence is shown along with ones with two different values of  $M_{\Omega}$  and the evolutionary tracks for sequence B. In a cluster of stars similar to the Sun one might expect to find stars scattered throughout the region between the non-rotating main sequence and one of the rotating ones. A typical spread at a given  $T_c$  is 0.2 to 0.3 in  $\log L$ . This spread of 0.75 mag is not observed in Praesape<sup>14</sup>, for instance.

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### Oblateness of solar models with rotating cores

Rood and Ulrich1 have discussed the oblateness of solar models with a rotating core. They find a larger oblateness for the same model than we did2. We have looked again into the oblateness calculation and now believe that the correct solution of the equations of Goldreich and Schubert3 gives an oblateness for the final model of our2 sequence C4 in agreement with the value derived by Rood and Ulrich. We also agree with Rood and Ulrich's explanation for the discrepancy.

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#### Geomagnetism and the tropospheric circulation

In a recent article in Nature1, King draws attition to certain similarities between the atmospheric flow utern in midtroposphere and the Earth's magnetic field. He stulates "some unknown magnetic-field dependent mechanism which exerts a control on the tropospheric circulation and acciated atmospheric pressure field. The evidence on which hoases this idea consists of the similarity between the distribution of the height of the 500-mbar pressure surface in the atmosphre and that of the geomagnetic field strength, particularly in latude 60°. He supports this with some indication that the features of the 500mbar surface have moved westwards in parallel wh the features of the geomagnetic field.

The purpose of this communication is to poir out that it is unnecessary, and probably misleading, to postulte any causal relationship between the geomagnetic field andthe 500-mbar contours, because the main features of the mar of 500-mbar contours can be explained by direct calculation rom physical and dynamical principles without consideratio of magnetic effects. Moreover any westward movements of theeatures of the

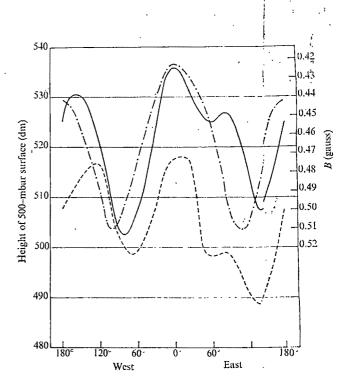


Fig. 1 Longitudinal variation of 500-mbar heightand magnetic field strength along latitude 60°N in January. — Observed 500mbar height from ref. 6; --- dynamically computed 500-mbar height; ---- magnetic field strength from Fig. 2 of ref. 1.

500-mbar height field appear to be merely the result of temporary fluctuations, and therefore unlikely to belinked with the westward motion of geomagnetic features which has continued over a century or more.

In recent years a great deal of effort has been devoted to the numerical solution of the equations of motion of the atmosphere, together with the thermodynamic equations, equations for radiative transfer and the mathematical representation of the other physical processes in the atmosphere. Given the external heat sources—the solar radiation—and appropriate thermal and mechanical boundary conditions at the Earth's surface, the aim has been to calculate the global atmospheric circulation and such related features as the distribution of temperature and pressure. The atmospheric circulation is far from being steady,

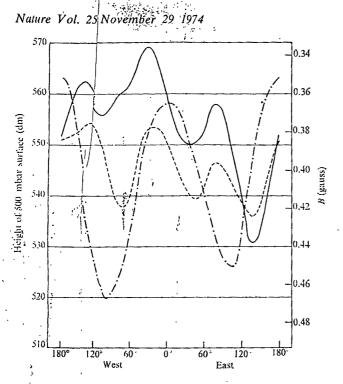


Fig. 2 Lonitudinal variation of 500-mbar height and magnetic field strengtl along latitude 40°N in January. (Explanation as Fig. 1.)

and the shor period disturbances—the depressions and anticyclones—play an essential role in maintaining the average circulation. The numerical calculations reproduce this feature. Starting from an arbitrary initial state, and integrating with respect to time, they reproduce the irregularly fluctuating character of the atmosphere. The mean seasonal circulation can be obtained by averaging over a period of some 30 d or more. A considerable degree of success has been obtained in such calcu-

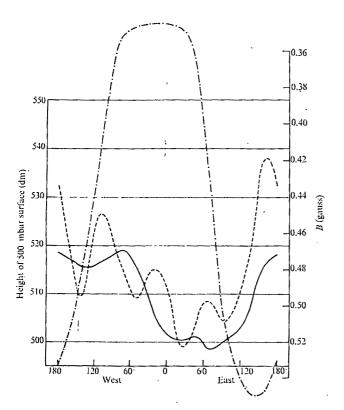


Fig. 3 Longitudinal variation of 500-mbar height and magnetic field strength: along latitude 60°S in July. (Explanation as Fig. 1 except that observed 500-mbar height is taken from ref. 9.)

lations and full reports have been published by several independent research groups<sup>2-4</sup>.

To illustrate this, I have taken the calculated profile of 500-mbar height around latitude 60°N from an integration performed during the course of one such numerical experiment with the model of Gilchrist, Corby and Newson<sup>5</sup> and plotted it in Fig. 1 along with the observed January profile as mapped by Heastie and Stephenson<sup>6</sup>. All the main features are fairly well reproduced although there are some small mislocations and incorrect intensities of the troughs and ridges. (The geomagnetic field strength B at 400 km is reproduced from ref. 1 for comparison.) It is noteworthy that the dynamical calculations reproduce the weak ridge at 80°E whereas there is no corresponding feature in the geomagnetic field. It is also noteworthy that the 500-mbar profile around latitude 40°N is also reproduced fairly well by the calculations (Fig. 2) although here

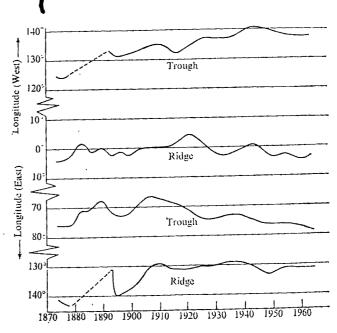


Fig. 4 Longitude of the main 500-mbar troughs and ridges at . 500-mbar as a function of time based on 10-yr running means (December-February).

the dominant wave number is three and not two as in the geomagnetic field.

Figure 3 shows similar profiles for latitude 60°S, also in winter. Here the observations are probably inadequate to define the observed field with any reliability but the agreement between calculated and observed distribution is such as to suggest that a geomagnetic effect need not be invoked to explain the main features of the profile and in particular the dominant wave number one.

King<sup>1</sup> uses a comparison of two sets of climatological charts as evidence for the westward movement of the features of the 500-mbar height field between the 1930s and 1950. The data available for the preparation of the earlier charts does not really warrant such a comparison, indeed the second set were prepared as a replacement of the earlier set when more data became available, and I am sure that the authors of the earlier charts would attribute the differences to lack of data rather than to any real change. More recently, in the course of research in long-range forecasting, a set of monthly charts of the 500-mbar heights for each year since 1874 has been built up in the Meteorological Office. In the earlier periods, when data for the

500-mbar level were lacking, these charts are obtained by a correlation procedure based on the surface pressure chart for which adequate observations are available. Figure 4 shows the changes in the longitude of the main 500-mbar troughs and ridges as a function of time. This diagram is based on 10-yr running means for the winter period (December-February). Although there is some confirmation of a westward displacement of these features (except the East Asiatic trough) in the period leading up to 1950, the pattern is one of irregular movements during the whole period, without much overall displacement. On the other hand the movement of the geomagnetic features has been believed to be westwards (albeit irregularly) at least since the early nineteenth century<sup>7,8</sup>. It does not, therefore, seem reasonable to seek an association between the erratic movements of these atmospheric features and the more or less steady progression of the geomagnetic features.

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DR KING REPLIES—It is widely believed that long term changes in the circulation of the lower atmosphere will be accompanied by climatic variations. The causes of these changes have not yet been established and at the present time, therefore, they are not predictable. I and other workers (see ref. 1) have suggested that the Earth's magnetic field may influence, through some unknown mechanism, the behaviour of the lower atmosphere at high latitudes in winter. Examples showing the type of evidence on which this suggestion is based are shown in Fig. 1; further evidence will be published elsewhere. I pointed out that such magnetometeorological evidence "cannot be construed as proof that the Earth's magnetic field and the average tropospheric pressure patterns are related, but it does suggest that more work on this problem should be undertaken.'

Sawyer<sup>2</sup> claims that "it is unnecessary, and probably misleading, to postulate any causal relationship between the geomagnetic field and the 500-mbar contours . . . " Several major objections can be made to this claim:

(1) It is based on the belief that the Meteorological Office model yields theoretical results in such good agreement with observations that it is unnecessary to postulate the existence of hitherto unconsidered relationships which might exist between the lower atmosphere and, for example, the geomagnetic field. Not all atmospheric modellers, however, would accept that current models yield such satisfactory results. Smagorinsky3, for example, says in the introduction to a GARP volume describing the 19 meteorological models currently known to exist: "All present models are sufficiently different from the real atmosphere so that, when real data are used with any one of them ... the conclusions are highly model-dependent." He continues: "The traumatic response of a model to alien data, for example conditioned by another model or, even worse, by the atmosphere itself, may often take the form of outright rejection of the

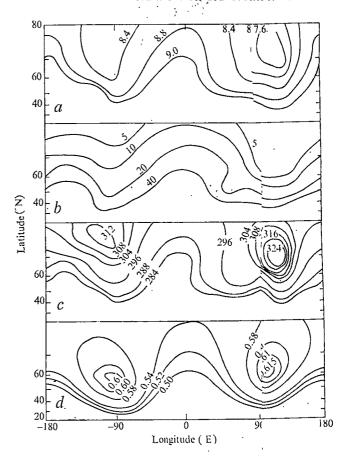


Fig. 1 a, b, c, Northern Hemisphere average rado refractivity maps, compiled by Bean et al. 10 from radiosade data for November 1958-62. a, Radio refractivity 'dry tern' tropospheric scale height (km); b, mean sealevel radio refractivity, 'wet term'; c, mean sealevel radio refractivity, 'dry term'. Tie maps effectively illustrate the spatial variations of, respectivey, the average tropospheric temperature, sealevel humidity and sealevel air density. d, Variation of total magnetic intensity (c.g.s. units) at the Earth's surface11.

transplant attempt." These statements indicate that even the best of the available models do not produce results in satisfactory agreement with the real atmosphere. Manab: and Terpstra4, after a major study carried out using the general circulation model developed at the NOAA Geophysical Huid Dynamics Laboratory, concluded that as far as the Northern Hemisphere is concerned: "The agreement between the distributions of geopotential height of the 500-mbar level surface in the mountain-model atmosphere and that in the actual atmosphere is particularly poor in higher latitudes." These are the latitudes for which I suggested that the geomagnetic field may exert a controlling influence on the tropospheric circulation1. As far as the Southern Hemisphere is concerned. Manabe and Terpstra4 reported: "The simulation of the flow field has much to be desired" and "The agreement between the computed and observed (pressure maps) is poor". The results of the calculations were judged to be "not very realistic" because "the intense surface westerlies in the middle latitudes of the Southern Hemisphere are almost missing" in the results obtained using the mountain model.

Some measure of the disagreement can be obtained by noting that the correlation coefficient between the observed and calculated 500-mbar height values at 60°N in January (from Fig. 1 of ref. 2) is only 0.69. The correlation coefficient between the observed 500-mbar data (shifted 25° westwards in order to allow for the height-dependent phase shift of the pressure variations) and the total intensity of the Earth's magnetic field at the Earth's surface is, however, -0.90, while that between

the January 50(mbar heights (phase-shifted) given by Palmen and Newton<sup>5</sup> an the intensity of the magnetic field for 60°N is -0.963. Such |gh correlation coefficients are very unusual and, although tey do not prove anything, their implications should not be dmissed lightly. The modelling work referred to above clearly sggests that the present understanding of the atmosphere emodied in the models is inadequate and that further interdisiplinary research is needed to discover what has been omitted from the models.

(2) Sawyer suggests that the numerical work which he refers to is based only on physical and dynamical principles and that it does not, theifore, take into account magnetic effects. This is not necessaril true; the results were obtained using the model of Gilchrist eal.6 which incorporates, as a necessary boundary condition, vales of the observed sea surface temperature which were bld constant throughout the calculations. The temperature rap used by Gilchrist et al. is such that the 6° C isotherm movs 21° towards the north (from 45°N to 66°N) while crossing the Atlantic from America to Europe and 18° northwards wile crossing the Pacific from Asia to America. It is obviously not possible to use the results of calculations which incorporate such boundary conditions to decide whether the Earth's mgnetic field or any other external phenomenon influences the circulation of the atmosphere.

(3) Sawyer clams that the main features of the 500-mbar maps are explained by numerical modelling; one feature of the maps which numerical work clearly cannot yet explain, however, is that they charge on a climatic time scale. Present thinking on the usefulness of numerical modelling in climatic studies is contained in ef. 7. This report, prepared for a session of the Joint Organisng Committee for GARP, says: "It is evident that detailed mathematical simulation of the atmosphere's three-dimensional behaviour cannot be the sole avenue to the study of long period variations of climate (for example, a period of the order of a decade or longer) and that the structure of the current general circulation models is unsuitable for that purpose."

McIntosh and Thom8 have summarised the current usefulness of numerical studies for climatic purposes as follows: "The synoptic and analogue methods of 30-day forecasting do not appear capable of being developed further to any significant extent. It is scarcely an exaggeration to say that in all other respects the meteorologist has, at present, nothing positive to contribute concerning future long range weather prospects. This situation is very unlikely to change until numerical studies of the general circulation are much further advanced than now."

These two quotations draw attention to the need for more knowledge about the processes which will alter the circulation of the atmosphere. One way of finding whether the magnetic field and the weather are related is to undertake, as I suggested1, "further comparisons of meteorological data from widely spaced epochs for which reliable maps of magnetic field strength are also available". Such work will require reasonably accurate magnetic information because the movement of features of the magnetic field pattern are by no means uniform. The manner in which the pattern changes is in fact extremely complex9 and Sawyer's representation of the motions of the geomagnetic anomalies as a "more or less steady progression of geomagnetic features" which are unlikely to be associated with the observed irregular secular movements of atmospheric features is unjustified.

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#### Initiation of the proto circum-Antarctic current

THE planktonic foraminiferal species Guembelitria stavensis Bandy (Fig. 1) lived in the austral gulf sea between Australia and Antarctica during the Oligocene, and with the final separation of the two continents at the eastern hinge of the South Tasman Rise, it spread out eastwards into the south-western Pacific. The palaeogeographical distribution and the very short stratigraphic range of G. stavensis in the south-western Pacific provides the first palaeontological evidence of the initiation of the proto circum-Antarctic current in the lower part of the Upper Oligocene.

It has been suggested that the circum-Antarctic current first became operative during either the late Oligocene1 about 30 Myr BP, the Middle Oligocene<sup>2,3</sup>, or the Lower Oligocene<sup>4</sup>. The first two suggestions were based apparently on interpretations of a late Palaeogene regional unconformity in the Tasman and Coral seas and the third was based on an interpretation of Oligocene planktonic Foraminifera from New Zealand.



Fig. 1 Guembelitria stavensis Bandy, × 560; site 276 DSDP Leg 29 (latitude 50° 48.11'S; longitude 176 48.40'E).

<sup>&</sup>lt;sup>1</sup> King, J. W., *Nature*, **247**, 131 (1974).
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<sup>&</sup>lt;sup>3</sup> Smagorinsky, J., Modelling for the first GARP global experiment, World Meteorological Organisation GARP Publications Series No. 14 (Geneva, 1974).

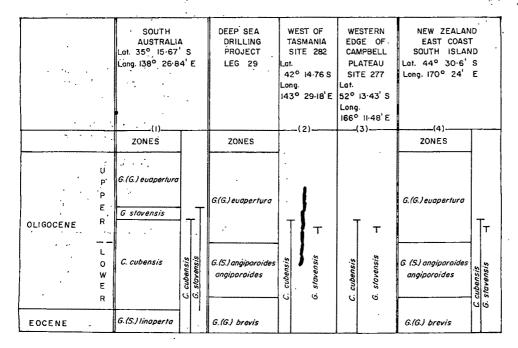


Fig. 2 Stratigraph records of G. stavensis in SouthAustralia, two sites of DSDP Legland one from New Zeand.

The palaeontological dating of the unconformity is basic to the original hypothesis. According to Kennett et al<sup>1</sup>. "The unconformity is centred approximately in the Globigerina angiporoides and Globigerina brevis foraminiferal zones<sup>9</sup> but frequently includes the Globigerina linaperta to Globigerina euapertura zones<sup>9</sup>; or the Isthmolithus recurvus to Reticulofenestra placomorpha zones<sup>10</sup> (late Eocene to Middle Oligocene)." But calcareous nannofossil I. recurvus—R. placomorpha zones have been correlated with the G. (S.) linaperta to G. (S.) angiporoides angiporoides zones in New Zealand<sup>5</sup>, that is the nannofossil zones are older than the G. (G.) euapertura zone and the erosion which formed the unconformity must also be older than that zone.

The stratigraphic and palaeogeographic distribution of *Guembelitria stavensis* which was originally described from the Middle Eocene of Alabama seems to fix the stratigraphic position of the initiation of the proto circum-Antarctic current.

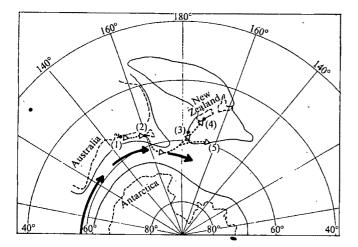


Fig. 3 Tentative migration route of G. stavensis (heavily dashed line) after the initiation of the proto circum-Antarctic current during the Upper Oligocene lowest G. (G.) evapertura zone. The shapes of continental areas (solid lines) are based approximately on the present 4,000 m bathymetric contour<sup>10</sup> (dashed lines show present continental boundaries). Details of present-day localities (1)—(4) are given in Fig. 2. Locality (5) is at latitude 50° 48.11'S; longitude 176° 48.40'E, DSDP Leg 29, site 276. Heavy arrows, proto circum-Antarctic current.

Guembelitria stavensis lived in the southern arts of South Australia during Lower to Upper Oligocene Giloguembelina cubensis – G. stavensis zones times<sup>6,7</sup> and becam extinct in the equivalent of the lowermost G. (G.) euapertura one (Fig. 2). It lived slightly longer there than in areas to theeast, however. Before it became extinct, G. stavensis migrated estwards and is recorded at this stratigraphic level west of Tasmaia at the Deep Sea Drilling Project (DSDP) Leg 29, site 282 Figs 2 and 3).

With the establishment of open sea condition following the separation of Tasmania and its southern continental extension from Antarctica, G. stavensis apparently spreal rapidly eastwards. It is recorded for only a short interval inthe lowest part of the G. (G.) evapertura zone on the western edge of the Campbell Plateau at DSDP site 277 (Figs 2 and 3. Guembelitria stavensis has also been encountered at DSDP site 276 on the eastern edge of the Campbell Plateau (latitule 50° 48.11'S; longitude 176° 48.40'E) and was obtained from a bit-sample presumed to be from the same stratigraphic levil in the G. (G.) evapertura zone. G. stavensis has also been recorded at the same stratigraphic level on the eastern coast of South Island, New Zealand (Figs 2 and 3) but it has not been recorded either north of this locality in the New Zealand Oligecene, or in the Tasman Sea sediments. A southward migration is therefore

I postulate that the migration of G. stavensis eastwards from the region of South Australia into the South Tasman Sea and south-western Pacific was permitted by the initiation of the proto circum-Antarctic current. The relatively short stratigraphic range of G. stavensis at sites east of South Australia appeared and became extinct just before the extinction of C. cubensis Palmer in the lowest G. (G.) euape-tura zone—can be placed on the radiometric scale, Berggren<sup>8</sup> has recorded the extinction of C. cubensis, termed the 'Chiloguembelina extinction Datum', in about the middle of the Oligorene Globigerina angulisuturalis/Globorotalia (Turborotalia) opima opima zone which has been correlated with the type Chatian. Records of K/Ar dates on the type Chattian<sup>8</sup> of the North German Basin give a range of 28.8-31.2 ± 1.5 Myr BP, and Berggren<sup>8</sup> has placed the extinction of C. cubensis at 28 Myr. Supporting evidence for this date has come from an examination of basalts in Victoria, Australia<sup>9</sup>, similarly dated at 26.5-27 Myr BP, which were interpreted as having been emplaced somewhat later than the locally recorded extinction of C. cubensis.

Thus, accepting the validity of the radiometric dates and the correlation of the extinction level of C. cubersis, the initiation

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of the protobircum-Antarctic current probably occurred between 27 an 28 Myr BP and that event was followed by the eastward migraon of G. stavensis.

I thank C. J. Fleming, J. Kennett, K. Swanson, and L. Leonard for ho and comments, and N. de B. Hornibrook for providing acces to the New Zealand record of G. stavensis.

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#### Advance of the Greenland Ice Sheet on to north-eastern Ellesmere Island

GEOLOGICAL evidence suggests that an important restricted ice advance occurred along north-western Greenland and north-eastern Ellesmere Island during the last glaciation.

Information on past ice margins is critical to the understanding of ice cores, delimitation of refugia, and preservation of both glacial and marine features that span several glaciations. It is widely assumed that during the last glaciation the Greenland Ice Sheet reached north-eastern Ellesmere Island1,2. It has also been assumed that during the last glaciation the ice sheets over northern Ellesmere Island and north-western Greenland coalesced to form a ridge 700-800 km wide centred over Kane Basin with outlet ice draining to the north-east and south-west along the line of Nares Straits. As yet, however, little geological evidence has been collected along these ice margins in relation to ice cores, and important existing literature has been ignored4.5.

North-eastern Ellesmere Island and north-western Greenland are separated by a distance varying from only 40 km to 130 km (Fig. 1). On north-eastern Ellesmere Island a major centre of ice dispersal exists over the United States Range where piedmont glaciers descend southwards along the northern edge of the Hazen Plateau. The southern margin of the active, United States Range Icefield is only 150 km to the north-west of glaciers from the Greenland Ice Sheet. The Hazen Plateau extends another 75 km to the south-east where it terminates directly across from Polaris Promontory, north-western Greenland. Physiographically, therefore, the region around the Hazen Plateau and the Polaris Promontory represents a relatively open area for the former interaction of the Ellesmere Island and Greenland ice sheets (Fig. 1).

Taylor<sup>6</sup> proposed that northern Ellesmere Island was once covered by the Greenland Ice Sheet, but this was rejected by Smith<sup>7</sup> on the basis of the distribution of Ellesmere erratics near the summits of the United States Range (Fig. 1). Christie8, however, noted the presence of red granite and gneiss erratics on Judge Daly Promontory and along the eastern edge of the Hazen Plateau, and suggested that a minor extension of the Greenland Ice Sheet on to north-eastern Ellesmere Island occurred at some unknown time in the past (Quaternary or Upper Tertiary glaciations?).

Red granites are exposed in Inglefield Land, north-western Greenland, and also amongst the moraines of Polaris Promontory8 and the glacial deposits south of Independence Fiord, in north-eastern Greenland. The hypothesis that the red granites were deposited by the Greenland Ice Sheet, which was possibly confluent with the south-eastward moving Ellesmere Island Ice Sheet over the eastern edge of the Hazen Plateau, implies that the granite erratics fall within a contained boundary. No detailed information yet exists, however, on the regional presence or absence of granite erratics over larger areas of northern Ellesmere Island.

Granite and gneiss bedrock has also been observed on the extreme northern coast of Ellesmere Island, and additional outcrops may extend inland beneath the United States Range Icefield. These would then coincide with the maximum, measured Pleistocene ice thicknesses over northern Ellesmere Island; and the southward movement of ice7 may thus have deposited these erratics on the outer Hazen Plateau and Judge Daly Promontory. This alternative hypothesis of a northern Ellesmere Island provenance is speculative and does not preclude the possibility that the Greenland Ice Sheet could have deposited the erratics8. Assuming that the red granite erratics on Ellesmere Island are products of the Greenland Ice Sheet then the remaining question concerns the timing of this ice advance.

Along the southern margin of the Hazen Plateau a discontinuous system of moraines marks the terminal position of ice draining out of the United States Range during the last glaciation<sup>10</sup> (Fig. 1). This system, termed the Hazen Moraines<sup>10</sup>, is associated with a maximum date of  $8,130\pm200~\text{BP}$  and is correlative with the Cockburn Stade of eastern Baffin Island11. Another till, of unknown age, extends above and beyond the Hazen Moraines and was deposited by an advance of the north-eastern Ellesmere Island ice out to Robeson Channel (Fig. 1). Shells adjacent to a coastal moraine, which is correlated with this till, date at 27,950±5,400 BP. This date, and the advanced surface weathering on a nearby ice-contact terrace, suggest that the advance may predate the last glaciation on northern Ellesmere Island. This most extensive northern Ellesmere Island ice advance is referred to as the Defosse Glaciation10. The preservation and lack of overriding of its coastal moraine and ice-contact terraces, suggest that any advance of the Greenland Ice Sheet on to north-eastern Ellesmere Island would have to predate the Defosse Glaciation.

In Inglefield Land (Fig. 1) there are three distinct glacial advances4. The oldest till (Stage 1) is the most extensive and reaches out to Kennedy Channel. It is characterised by ironstained, silicate rocks, and limestone boulders with solution cavities. The Stage 1 till is possibly correlative with the advance of the Greenland Ice Sheet on to north-eastern Ellesmere Island<sup>4,8</sup>. The Stage 2 till, less extensive than the Stage 1 till, is oxidised to a depth of one metre. Stage 3 defines the least extensive and most recent ice advance on Inglefield Land and is marked by a semicontinuous moraine system. A large terrace, possibly contemporaneous with, or older than, the Stage 3 ice advance, contained an organic layer dated at  $20,800\pm2,900$  BP. The distribution and relative ages of the three glacial stages on Inglefield land, therefore, indicate that a minor advance took place in this locality during the last glaciation and that the advance of the Greenland Ice Sheet on to north-eastern Ellesmere Island would have to be as old as Tedrow's Stage 1

Davies also notes at least three glaciations on northern Greenland during the Pleistocene<sup>5</sup>. The oldest glaciations are believed to be pre-Wisconsin in age and successively less extensive toward the present. Two stages of glaciation occurred on Polaris Promontory and again the oldest is the most extensive and is considered to pre-date the latest Würm-Wisconsin glaciation<sup>5</sup>. Karlstrom also suggests (personal communication) that low levels of glacial intensity occurred during the last major glaciation over Polaris Promontory. He adds that the Greenland ice may have approached, but not merged with, the Canadian ice during the

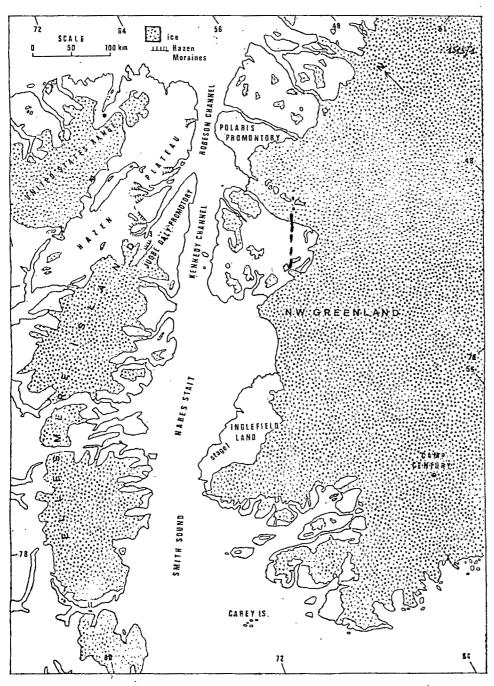


Fig. 1 Mp of area covered by the Greenland IoSheet.

last one or two major glaciations. The restricted position of the Hazen Moraines, and the preservation of the features from the Defosse Glaciation on north-eastern Ellesmere Island, supports that suggestion.

Bendix-Almgreen et al<sup>12</sup>, working on the Carey Islands, 110 km off the coast of north-western Greenland (Fig. 1), conclude from the distribution of erratics that the Greenland Ice Sheet formerly extended out to and beyond that area. They<sup>12</sup> suggest that Nares Strait was probably completely filled with the Greenland and Ellesmere Island ice sheets during the full-bodied stage of the Wisconsin. The date of deglaciation of the Carey Islands is not known although it may predate the age of shells from Saunders Island, 25 km off the coast of north-western Greenland, which date at more than 32,000 years BP (ref. 13). This date is probably a minimum age estimate on the last retreat of the Greenland Ice Sheet from Smith Sound. The available geological evidence and climatic considerations<sup>14</sup> along Smith Sound, Nares Strait and Kennedy and Robeson channels does not support the conclusions

that towards the end of the last glaciation the north-western Greenland Ice Sheet was 1,300 m thicker than today<sup>15</sup>.

High coastal marine limits (>120 m) have also been interpreted as evidence for an extensive ice advance in that region during the last glaciation. The interpretation is, however, based on the assumptions that the marine limits are all postglacial features and that all these features represent a glacio-isostatic response related to a very extensive ice advance. As yet, however, there are no radiocarbon dates on these high coastal marine limits and the observed postglacial uplift over north-eastern Ellesmere Island and north-western Greenland can be reproduced by an uplift model<sup>16</sup> using the restricted ice margins discussed in both areas<sup>5-10</sup>.

Present evidence indicates that detailed records of multiple glaciations and sealevel changes are preserved along the coastlines of Smith Sound, Nares Strait and Kennedy and Robeson channels. There should, therefore, be stratigraphic evidence on the magnitude and age of the proposed advance of the Greenland Ice Sheet on to north-eastern Ellesmere Island

as indicated if the preservation of the oldest, subsequent ice advances in bh areas. The same information applies to the stratigraphic d chronological clarification of the proposed Greenland-Elimere Island ice-ridge over Kane Basin.

At present he advance of the Greenland Ice Sheet on to north-easternillesmere Island is considered inconclusive and, if it did occul it would have to pre-date the late Wisconsin, and possiblythe entire Wisconsin glaciation. The possible presence of lisconsin refugia on northern Ellesmere Island<sup>17</sup> is considered on sistent with the geological evidence 10.

Evidence bm glacial geology is also critical for the reconstruction of se time profiles through existing and future ice cores in the reion as such data will affect directly the parameters in the flow midels chosen. If the ice sheets in the High Canadian and Greenlad Arctic were relatively inactive during the last glaciation or laciations then the ice cores obtained from these areas may bolder than originally expected. Sections in the ice cores18 tat indicate conditions colder than the present may coincide ith glacial inactivity which resulted from a more continental emate, whereas the warmer sections in the cores may reflect mre maritime (glacial) conditions. The chronology of glacial activity during the late Quaternary is, therefore, an important sorce for both the dating and palaeoclimatic interpretation of te ice cores.

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#### **Exfoliated pebbles** and sheeting in the Triassic

SHATTERED pebbles and sheeted bedrock are common weathering phenomena in most modern deserts1-3 but their existence in ancient desert environments does not seem to have been described. This communication documents their occurrence in South Wales, where they were formed during the Triassic period, some 200 Myr ago.

Two facies are developed in the Upper Triassic of South Wales4.5-the Keuper Marl, a purplish-red dolomitic silt, which is considered to have formed in a hypersaline lake or inland sea6-8, and the 'littoral' or marginal Triassic (wherein the weathering phenomena occur), red beds of variable lithology and facies, which occur below and laterally equivalent to the Keuper Marl. Environments and landforms represented by the marginal facies in Glamorgan include alluvial fans9 and plains, pediments, wadis10, playas and soils. The deposits of these environments (fanglomerates, calcarenites, playa silts, lacustrine cryptalgal laminites, fenestral (birdseye) limestones and calcretes) accumulated against and below a retreating Palaeozoic mountain front and around monadnocks ("islands",4,5) mainly composed of Carboniferous Limestone.

Many limestone pebbles on the surface of a conglomerate, exposed at Hayes Point near Barry (Grid Ref. ST143674) are completely shattered (Fig. 1). Some pebbles have fractured to a perfect onion-skin pattern, consisting of concentric spheres of rock, split a few millimetres apart. Other pebbles have simply shattered into several angular slices. Cracks in pebbles are infilled with red silt and sand, except where the cracks have penetrated a few centimetres below the former land surface, when they are now filled with calcite. The conglomerate (110 cm thick) is a moderately sorted mixture of limestone pebbles and sand, with an irregular and deeply scoured base. It was clearly deposited relatively quickly (hours or days) and is probably the product of stream flooding. A prolonged period of weathering followed deposition of the conglomerate, when the surface pebbles were shattered and exfoliated.

At Bendrick Rock (Grid Ref. ST131668) the Triassic red beds abut against and then overlie Carboniferous limestone which must have formed a small monadnock or knoll, rising out of



Fig 1 Exfoliated pebble of Carboniferous limestone, with concentric (onion-skin) fractures, in red Triassic sandy dolomite. Hayes Point, Glamorgan.

the Triassic pediment. The surface of the Carboniferous limestone, down to 30 cm, is split into thin sheets, parallel to the former surface. Red silt, probably of aeolian origin, occurs between the sheets. Fossils and ooliths in the limestone are truncated by the cracks. The sheeting clearly developed upon the former surface and not within a weathering profile. Other features at and just below this weathered surface include neptunian dykes and an iron-staining pattern around joint blocks in the Carboniferous limestone. Calcretes (of which at least 12 occur) interbedded with red marls are developed against the Carboniferous limestone monadnock on which the sheeting is developed, and probably formed at the same time. Calcretes (pedogenic limestones) represent periods of soil formation which lasted the order of 5,000 to 30,000 yr11. This suggests that the sheeting of the bedrock surface developed during a considerably longer period of time (104 to 105 yr).

Much debate has been concerned with the origin of shattered pebbles and exfoliated surfaces in modern deserts1.3, Experiments by Blackwelder<sup>12,13</sup> and Griggs<sup>14</sup> demonstrated that a purely physical process of repeated temperature changes did not result in rock disintegration, but that some degree of chemical weathering (even just the presence of water) was required. Records<sup>15,16</sup> of fractured pebbles of nearly chemically inert flint and quartzite do, however, suggest that insolation alone can be effective. The occurrence of frost-weathering in deserts has been realised only recently and could also be an effective cause of rock shattering, especially in higher altitude deserts, during cooler climatic phases1,17. Crystallisation of salts within confined spaces18 (such as cracks along joint, cleavage and bedding planes) leads to rock disintegration. This is a common phenomenon in arid (and coastal) environments19,20, particularly on saline playas, along alluvial fan channels21 and on high tidal flats22. Precipitation of calcite during surface calcretisation can have a similar rock-splitting effect<sup>23</sup>.

In these Triassic examples of exfoliation, there is no evidence of contemporaneous chemical weathering, such as karst and solution structures, although pebbles and rock sheets are commonly dolomitised (probably a post-Triassic diagenetic event). No evidence has been found for the presence or former existence of salt deposits or their replacements at the exfoliated horizons, although salt efflorescences could conceivably have been dissolved following deposition of the overlying beds. Evaporites do occur higher in the succession (some 100 m above) towards the top of the Keuper Marl. Salt weathering, however, seems to produce linear fractures and flakes of rock21,22 rather than the concentric type of fracture illustrated in Fig. 1. Calcite of calcrete (pedogenic) origin does not occur within cracks—these are filled with clastic sediment or sparite druse. It would seem then that the exfoliation structures formed primarily through isolation, although water (from precipitation) or dew24 may have been a contributory factor. In view of the Rhaetic marine transgression towards the end of the Triassic period, it is likely that this part of South Wales was a low altitude desert area during this time, where frost action would rarely occur.

The presence of exfoliated pebbles and sheeted bedrock is evidence additional to that provided by aeolian dune-bedding, millet-seed sands, ventifacts, calcretes and evaporites for an arid or semi-arid climate during the Triassic period in Britain. Paleomagnetic data support this deduction by indicating a low palaeolatitude (10-30 °N) for Britain during the Triassic25.

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#### Diverse microfossils in Precambria Onverwacht group rocks of South Ifrica

We report the discovery of some complex micofossils from the Kromberg formation of the Onverwacht goup of South Africa. The samples, in which the microfossil were found, were collected by D.O.H. along the banks of the Komati River in the Barberton Mountain Land, at Skapbrug, near the old JCI mining camp. The Kromberg fomation lies in the Upper part of the Onverwacht group, above the Middle Marker Horizon, which has been dated as  $3.35 \times 10^9$  yr old<sup>1</sup>. The Onverwacht group is the oldest member ofthe Swaziland Supergroup and lies below the  $3.1 \times 10^9$  yr old: ig Tree group. Microfossils have been reported from the FigTree group2,3, and simple spherical and filamentous forms from the Onverwacht4-6. Spherical and cup-shaped carbonceous microstructures have been reported7-9 from the Overwacht but were not regarded as biogenic in origin.

The present forms are more complex than these previously described4-6; they were discovered in petrological thin sections and include spheroids, filaments and agglomerations of cells. Although spheroids have previously been decribed, those from the Kromberg formation<sup>5</sup> fell within a narow size range 15-20 µm (Fig. 1a). In the present samples, more cells of the same size have been discovered but smaller cills ranging in diameter from 3 µm to 7 µm have also been found (Fig. 1b), having a relatively thick wall (0.5-1 µm thick) and often black or very dark brown in colour. Although mainly found solitary and not associated with other organic matter, they are occasionally paired.

The filaments in the thin sections are of three types: (i) filaments similar in dimensions and morphology to those previously described<sup>5</sup>; (ii) small (diameter 1-3 μm, length up to 25 μm; Fig. 1c), non-septate filaments; (iii) segmented filaments (diameter 1.5-4  $\mu$ m, length up to 42  $\mu$ m, and with individual cells of diameter 1.5-4  $\mu$ m and length 2-5  $\mu$ m; Fig. 1d).

Large numbers of cells of uniform size (diameter 3-8 μm) occur associated, set in a matrix of amorphous organic matter, on average 5-10 µm apart (Fig. 1e).

In two samples, very rich in organic matter, we found what appears to be multicellular tissue. This is composed of very large numbers of cells of more or less uniform size (7-10 µm; Fig. 1f). These cells are approximately equidimensional and

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their overalbutline is modified by the presence of adjacent cells, that ishey appear to have walls in common with adjoining cells to distinct overall outline to these agglomerations of cells is dismable, the maximum diameters of single agglomerations rankg up to more than 200 µm. Some of the agglomerationshave a rounded outline, while others enclose areas of wateclear chert, free of organic matter.

The large/ariety of morphologies and the number of individual spimens leads us to the conclusion that the structures observe are biogenic in origin. Their state of preserva-



Fig. 1 a, Large spheroidal cell, diameter 15  $\mu$ m (sample 1; thin black chert from between pillows in lava). b, Small spheroidal cell, diameter 6 μm (sample 7; black chert). c, Small nonseptate filament, diameter 2 μm (sample 1A; black chert). d, Segmented filament, diameter 2 μm (sample 3; black chert, very rich in organic matter). e, Spheres (arrowed) set in matrix of amorphous organic matter, average diameter 4 µm (sample 1A; black chert). f, Agglomeration of cells, diameter 7 µm, having appearance of multicellular structure (sample 3; black chert, very rich in organic matter). Scale bar represents approximately 5 µm (all micrographs).

tion is variable, depending on the degree of metamorphism of the sample, but allowing for the carbonisation which has taken place<sup>5,10</sup>, the morphology of the fossils is distinct and can be compared with the morphologies of specimens from younger rocks11-14. The morphologies found—spheroids, single or paired, filaments, segmented or nonsegmented, 'colonial' structures where single spheroids occur associated, set in a matrix of apparently mucilaginous material, and multicellular structures—have all been described from prokaryotic groups such as the blue-green algae, and some of the bacteria.

There is ample evidence for the presence of biological activity in Archaean rocks. Stromatolites have been described from Rhodesia<sup>15</sup> in rocks now dated at 3.1 × 10<sup>9</sup> yr old<sup>16</sup>, and from the Canadian Shield17 in rocks more than 2.7 × 109 yr old18. Schopf et al.15 believe that the discovery of the Rhodesian stromatolites indicates the activity of blue-green algae but stromatolites have been described from hot springs19,20 in which photosynthetic bacteria play an important role. Doemel and Brock<sup>21</sup> were able to demonstrate that in the hot spring deposits at Yellowstone National Park photosynthetic bacteria control the lamination of the mat structure as a response to light fluctuations. The organism present is Chloroflexus, a filamentous photosynthetic bacterium which acts as a sediment trapper; it produces a laminated siliceous sediment in which the laminae are of variable thickness and where, under the regions rich in organic material, laminated silica is preserved. In the modern sediment, blue-green algae are also present to varying extents depending on the depth of the layers where light intensity may be the controlling factor. Chloroflexus cells are much more resistant to degradation than blue-green algae and bacterial remains are the most abundant. Although the blue-green algae virtually disappeared at low levels in the mat, however, they could still have played a role in its formation. Their results21 "show that stromatolites might have been formed solely by filamentous photosynthetic bacteria".

All the Onverwacht cherts studied from the Kromberg formation are finely banded with bands rich in organic material alternating with those which are poor in such. This forms the bulk of the rock studied, even when no structurally preservable microfossils can be observed. Such banded rocks are generally the result of biological activity, whether they be bacterial or algal mat structures, or glacial varves.

Thus, although the present discovery of microfossils supports the hypothesis that quite diverse remains of biological activity can be detected in the rocks of the Onverwacht group, there is at present no way of determining their biological affinities.

In light of recent work with photosynthetic bacterial stromatolites and mat structures it is possible that the microfossils we are examining are the remains of a similar type of mixture of organisms in the modern laminated mats.

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#### Reliability of amino acid racemisation dating and palaeotemperature analysis on bones

THE general inability of isotope geologists to work out techniques for dating continental Pleistocene deposits has led to the conception of nonisotopic chemical methods. Hare and Mitterer1 noted that fossils could possibly be dated by determining the extent to which the l optical isomer of a given amino acid had racemised to form the d isomer which is initially absent in skeletal material. Obviously, the rate constant for the reaction must be known accurately if age is to be calculated, but its value is difficult to assess: it is highly temperature dependent<sup>2</sup> (about 20% ° C<sup>-1</sup>); it depends on the state of the amino acids (free amino acids racemise about an order of magnitude more rapidly than those bound as peptites)3-5; and it is highly dependent on environment<sup>6</sup>. Furthermore, free amino acids may back react to produce the bound forms, and there could be open system conditions during diagenesis2.

Bada et al. have investigated amino acid racemisation geochemistry as a method of dating fossil bones<sup>2</sup> and as a means of deducing palaeotemperature data where ages have been independently determined. They showed that 'ages' or 'palaeotemperatures' calculated from data of racemisation of amino acids in Upper Pleistocene bones are generally comparable to independently deduced values. That does support the notion that racemisation may be useful in geochronological and palaeoclimatic evaluations, but their results have many inconsistencies.

Bada et al.2 determined the extents of lacisation of amino acids in two or three year old bone fragmts collected in the Arizona desert. From the observed exteniotcemisation of glutamic acid, and the temperature dependen of the rate constants, it is possible to calculate that the saple was preserved at an average temperature of about 1.C. That is however, completely unlikely. A more reasonablexplanation for the anomalously large degree of racemision is that there is a brief interval after the death of the orgism, during which racemisation proceeds more rapidly that does later in the history of the fossil. If that is actually e case then 'ages' or 'temperatures' which are calculated y assuming constant racemisation, may be quite misleading.

Moreover, the rate constant, normalised to a /en temperature, is extremely sensitive to the environmentn particular, the extent of isoleucine racemisation (actually)imerisation) in bone from La Brea tar pits was found to be nch less than expected from data on isoleucine racemisatio on samples from Muleta Cave and elsewhere<sup>6</sup>. This is appantly because of the relatively anhydrous environment proded by the asphalt in which the La Brea sample was preserd.

Turekian and Bada<sup>7</sup> have established a hronological sequence in a section from Muleta Cave in allorca from radiometric and isoleucine racemisation ages (& latter were calculated by assuming that the temperature tere has been constant at 19° C and that the rate constant athis temperature is  $6 \times 10^{-6}$  yr<sup>-1</sup>). Their results show that amo acid dates and 230Th dates agree (the 14C dates are muchyounger, but that is probably because whole bone was analyse, rather than just the collagen fraction8). Bada9 has redeterined the rate constant, and his recent results give a value of  $16 \times 10^{-6} \text{ yr}^{-1}$ at 19° C (this value is also similar to that estimted using the rate constant for aspartic acid, calculated for the 8,600 yr old Muleta sample7, and the ratio of rate constant for aspartic acid and isoleucine racemisation2). Ages recaulated using this value of k are lower than previously calculate racemisation ages by more than a factor of two, and are inonsistent with <sup>230</sup>Th/U ages (Table 1).

Bada has also established a chronology from the Z section of Muleta Cave (Table 2). It is clear from thee results that the 14C ages on bone collagen fraction, the amino acid chronology, and the 230Th chronology are all strongly discordant. This apparent discordance becomes even greater when k is modified to account for the facts tha temperatures were generally lower than 19° C and that it was the collagen fraction, which racemises more slowly than thewhole bone<sup>10</sup>, which was sometimes analysed.

Table 1 Calculated age sequence from X sector in Muleta Cave*								
Position in section (cm)	Nature of sample	Dating technique	Calculated age (yr)					
80	Travertine	<sup>230</sup> Th	85,000 and 97,000					
95	Collagen fraction of bone	Isoleucine racemisation	180,000					
200	Collagen fraction of bone	Isoleucine racemisation	280,000					
250	Bone	Aspartic acid racemisation						
300	Whole bone	14C	16,400					
350-400	Whole bone	<sup>14</sup> C	18,735					

<sup>\*</sup> Isoleucine racemisation ages are calculated using  $k = 2.6 \times 10^{-6} \text{ yr}^{-1}$ .

Table 2 Calculated age sequence from section Z in Muleta Cave*								
Position of sample in section (cm)	Nature of sample	Dating technique	Calculated age (ग)					
350	Bone	14 <b>C</b>	14,500					
350	Bone	Isoleucine racemisation	32,000					
475	Bone	Isoleucine racemisation	26,000					
550	Collagen fraction of bone	Isoleucine racemisation	120,000					
550	Bone	Aspartic acid racemisation	34,000					
550-600	Collagen fraction of bone	14C	28,600					

<sup>\*</sup> Using  $k=2.6\times10^{-6}$  yr<sup>-1</sup>.

	Isoleucine	Glutamic	Alanine	Aspartic
	Leucine	Leucine	Glutamic	Leucine
Predicted by heating experiments <sup>2</sup> Muleta Cave (28,900 yr, <sup>14</sup> C age) Florisbad (38,600 yr, <sup>14</sup> C age) Border Cave (> 45,000 yr, <sup>14</sup> C age) Olduvai (~200,000-300,000 yr) Swartkyans (~2 Myr)	$\begin{array}{c} 1\\ 0.90\pm0.2\\ 1.3\pm0.2\\ 0.84\pm0.2\\ 1.1\pm0.2\\ 1.6\pm0.3 \end{array}$	$2-2.4$ $3.0\pm0.6$ $1.8\pm0.4$ $2.5\pm0.5$ $2.1\pm0.4$ $1.3+0.3$	$\begin{array}{c} 1 \\ 0.84 \pm 0.2 \\ 1.6 \pm 0.3 \\ 1.2 \pm 0.2 \\ 0.92 \pm 0.2 \\ 1.3 \pm 0.3 \end{array}$	8 5.8±1.0 7.3±1.0 4.8±1.0 2.5±0.5 2.6±0.5

<sup>\*</sup> Calculaterom the results of Bada et al.2, k by assuming the d isomer to be absent in modern specimens. Errors in the rate constant ratios are estimated:  $\pm 20\%$  using the analytical uncertainty ( $\pm 10\%$ ) in the gas chromatographic analyses of the d/l ratio (K. A. Kvenvolden, personal comunication).

Schroeder nd Bada<sup>10</sup> determined the extent of aspartic acid racemision in 14C dated bones (see also ref. 2), and used the relts to calculate palaeotemperature histories. Using reasorble assumptions they calculated that the temperature in Juleta Cave was about 4°C colder between 10,000 and 1000 years ago than it is at present, a conclusion consistent wa most other evidence. Using their data and approach to liculate the average Muleta temperature between 19,000 and 2900 yr BP (sample 1704A) it is possible to estimate that temperaires during that period were similar to those at present. Nost other evidence, including that summarised by Schroede and Bada, indicates, however, that during that period the temperature was much cooler than at present. The difference can be reconciled if it is assumed that the 14C age is wrong but such an assertion would undermine other conclusions.

Bada et al. determined rates of racemisation of amino acids in bones heatd at elevated temperatures in laboratory experiments. They jointed out that if a given sample has not been altered by opn system conditions, then the relative extent of racemisation of the different amino acids must be consistent with the relaive extents predicted from the rate constant data. That is an important observation because it allows a check on wheher or not the sample has been a closed system. The check can be applied by using the ratios of optical isomers for the various samples to calculate ratios of rate constants for the various anino acids, so that the results can be compared with those predicted from the high temperature heating experiments of Bada et al.2 (Table 3).

For all samples at least one of the calculated rate constant ratios is inconsistent with the predicted value (Table 3), so all of these samples have suffered some contamination. Different samples seem to be contaminated with different amino acids. In my opinior no meaningful conclusions of palaeotemperature or palaeoclimate can be deduced from any of these results.

The contribution from Bada et al.2 is very significant in that it outlines quantitative, a priori, criteria for determining if amino acid tacemisation ages or temperatures are reliable. Their findings, and the fact that reasonable ages and temperatures are sometimes obtained, indicates that the method has potential. It dearly faces many basic problems, however, and in my opinion no palaeoclimatic or geochronological inferences should be drawn from racemisation data until the basic geochemistry is thoroughly understood and the bases of the method firmly established.

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DR BADA REPLIES-Bender's review of my work is both inaccurate and incomplete. He has not cited two of my publications dealing with aspartic acid racemisation dating1,2. (Although one paper was only recently published, I sent Bender a preprint the first of this year when he informed me he was writing a review.) In those articles I show that after 'calibrating' the amino acid racemisation reactions using a radiocarbon dated bone, it is then possible to date other bones from the same site, which are either too old or too small for radiocarbon dating. The only assumption in this approach is that the average temperature experienced by the calibration sample is representative of the average temperature experienced by the other sample. Ages thus deduced are in good agreement with radiocarbon ages determined on the same samples2.

Bender points out that several factors (temperature, protein hydrolysis, and so on) can influence the extent of amino acid racemisation in a fossil. I am fully aware of these factors and have discussed the potential limitations of racemisation dating which arise from each of them<sup>3-5</sup>.

The bovine bones discussed by Bender were found lying on the surface in the desert plateau region of south-western Arizona3. They were collected because I wanted modern bone samples for kinetic studies of isoleucine epimerisation in bone. The samples seemed ideal, as they had already experienced the early phase of animal bone diagenesis (that is, carnivore and microbial consumption of the adhering flesh). The low alloisoleucine/isoleucine ratio (0.02) indicated the bones were not very old.

Following the isoleucine studies, an analysis of the extent of racemisation of aspartic acid in the desert bones was carried out, and this indicated a d/l ratio of 0.15 (ref. 4). An analysis of a piece of bone from a beef steak, however, yielded a d/laspartic acid ratio of 0.07 (ref. 1). That value is in close agreement with d/l ratios of aspartic acid found for several proteins carried through the same analytical steps as the bone samples<sup>6,7</sup>. A d/l ratio of 0.07 must, therefore, represent the t = 0 value for aspartic acid.

The aspartic acid results suggested that the desert bones were probably older than the 2-3 yr originally estimated. To calculate an average temperature to which the bones have been exposed, as Bender has done, in the absence of firm documentation of their age, is unwarranted. Furthermore, although the present mean temperature in the region where the bones were found (Yuma, Arizona) is 22° C (ref. 8) ground temperatures as high as 80°-85° C have been reported (M. A. Barker, unpublished). An older age and the occasional ex-

<sup>&</sup>lt;sup>1</sup> Hare, P. E., and Mitterer, E., Yb. Carnegie Instn. Wash., 67, 205 (1969).

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tremely high temperatures could account for the extent of racemisation in the Arizona desert bones.

Bender contends that racemisation rates in bone are extremely sensitive to environmental factors other than temperature. He states that Schroeder and myself<sup>9</sup> also noticed this; we have never made this statement and would, in fact, strongly disagree with it. The rate constants of aspartic acid racemisation determined from the extent of racemisation in radiocarbon dated bones younger than 10,000 yr (post-glacial), all show a remarkable correlation with the present mean temperatures of the general regions in which the bones were found<sup>1,2,9,10</sup>. The same correlation exists for glacial age bones, but this should be considered less reliable, as temperature changes during the last ice age may have been different at the various sites. The sites we have studied range from temperate coastal to near tropical mountainous regions in Africa, and from hot, arid rock shelters in Iran and Iraq to a limestone cave on Mallorca. If environmental factors other than temperature had an important influence on racemisation rates, it would seem that the correlation between the rate constants and the average temperatures for these various sites would not be as good as it is.

The racemisation rates<sup>4</sup>,<sup>11</sup> for the bones from the La Brea Tar Pits are indeed anomalous. This type of environment is, however, rare and extreme. Racemisation rates determined from radiocarbon dated bones from the surrounding southern California area would hardly be expected to be applicable to the tar pits. In order to date the bones in the tar pits, a 'calibration' should be carried out by determining the extent of racemisation in a radiocarbon dated bone from the tar pits, and then other samples from the tar pits could be dated.

The isoleucine ages published by Turekian and myself for Muleta Cave<sup>12</sup> were only preliminary results, to show the possible potential of the racemisation technique. Detailed kinetic studies at elevated temperatures have yielded better estimates of  $k_{1so}$  at the Muleta Cave temperature<sup>3</sup>, and these  $k_{iso}$  values have yielded ages in reasonable agreement with radiocarbon ages<sup>3,4</sup>. Also, the allo/iso ratios in the Muleta Cave bones were very small (less than 0.1) and difficult to measure accurately. The isoleucine ages were only rough estimates.

We have obtained aspartic acid racemisation ages for Muleta Cave (ref. 2, and J. L. Bada, unpublished) (Table 1). When the radiocarbon age is based on the collagen component, it agrees excellently with the aspartic acid racemisation age. The racemisation age determined for the bone from the X section is, however, much older than the radiocarbon ages obtained from that section. This is not surprising, as the radiocarbon ages were based on the total acid-leached carbon.

	Table 1 Muleta Cave ages										
Cawe section*	d/l Aspartic acid	Aspartic acid agef (yr)	<sup>14</sup> C age (yr)								
Z-400 cm (UCLA 1704D)‡	0.273		16,850±200								
E-350 cm (UCLA 1704E)	0.293	18,600	18,980±200								
Z-550-600 cm (UCLA 1704A)	0.455	33,700	28,600±600								
X-250 cm X-300 cm (SI-648) X-350 to 400 cm	0.73	69,000	16,335±415 18,735±555								
(SI-650)			•								

<sup>\*</sup> Notation in parentheses is radiocarbon laboratory identification number. The UCLA results are collagen dates, whereas the SI ages are for acid leached carbonates. †  $k = 1.25 \times 10^{-5} \text{ yr}^{-1}$ .

Such ages are often unreliable because of exchae processes involving ground water carbonates of diffint specific activities<sup>13</sup>.

The aspartic acid age for bone from the 250 cm rizon of the cave X section suggests an age of about 100,00r artits base (the total section depth is approximately 400 c. The <sup>230</sup>Th age of the surface of a stalagmite around which the section formed was found to be  $85,000\pm8,000$  yr (ref. 1. This age is consistent with the age of 100,000 yr for the base the section, as it seems likely that carbonate accumulation the stalagmite would stop shortly after sedimentation in the tion began.

Schroeder and I<sup>9</sup> did not use sample 1704A inur temperature calculations because of the uncertainties in themperature model for the Mediterranean area at 20,0000,000 yr BP. Also, the radiocarbon age of this sample is probal less reliable than that of the other radiocarbon-dated samplerom Muleta Cave, as it was collected from an horizon 50 cmick whereas the other samples were obtained from more disete horizons. Thus, the radiocarbon age represents an 'avege' over the 50-cm section. The racemisation analysis was tried out on a single 10 g piece which could have an age slittly different from the 'average' radiocarbon age.

Using the procedure outlined by Schroeder id myself<sup>9</sup>, I calculate that the difference between the averagtemperatures experienced by samples 1704C and 1702A (stable 1) is  $-0.8^{\circ}$  C, compared with the difference of  $-1.6^{\circ}$  experienced between 1704C and 1704D. Evidence<sup>9,14</sup> indicateshat there was a period of gradually increasing temperature between  $\sim 20,000$  and 50,000 yr BP, although temperatures never riched present values. Some of the coldest temperatures during he last glacial occurred at about 15,000–20,000 yr BP (ref. 1). Therefore, a bone of about that age (such as 1704D) wouldield a slightly cooler average temperature than a bone with ange of around 30,000 yr (such as 1704A).

Because the relative rates of amino acidracemisation determined by heating modern bone fragmens at elevated temperatures are not exactly the same as those i fossil bones, Bender believes that all fossil bones analysed b Kvenvolden, Peterson and myself4 were contaminated to some degree with modern amino acids. Although the Arrhenius ativation energies  $(E_a)$  are about the same for the variou amino acid racemisation reactions, they certainly differ by atleast tenths of a kcalorie per mol (the experimental uncertainties of the  $E_a$  values). This difference is not enough to chinge the order of racemisation rates (that is, aspartic acid > alanine \( \sime \) glutamic acid > leucine \( \Leftrightarrow \) isoleucine) in goingfrom elevated to lower temperatures, although it could causesmall changes in the relative rates. For example, if any two reations have  $E_a$ values of 33.0 and 32.0 kcalorie mol-1 and the ratio of the rate constants is 5:1 at 100°C, then at 20°C the ratio would have changed to 3.5:1. Thus, the relative rates of racemisation in uncontaminated fossil bones coud be different from those found in modern bones heated at elevated tempera-

Bender (his Table 3) shows that the relative racemisation rates for bones from Muleta and Border Caves are essentially identical (within experimental error). As these two bones differ considerably in age and in depositional environment, I suggest that this similarity, in fact, indicates that these bones are not contaminated and that the relative rates of racemisation observed represent the pattern expected in uncontaminated bones. Other samples in Bender's Table 3 are contaminated to various degrees, a point which I have emphasised before.

Bender seems to have missed the point that even if a bone is contaminated, racemisation can still provide a minimum age. Contamination introduces amino acids that are more modern (that is, consist of mainly the *l*-enantiomers) than the indigenous collagen-bound amino acids, and therefore lowers the *d|l* amino acid ratios.

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TFirst sample used for site calibration (see refs 1 and 2). Aspartic acid racemisation results taken from ref. 2 and J. L. Bada (unpublished).

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#### Theory ofliquid—liquid and liquid-va our equilibria

We formulat here a general molecular theory of solutions, which predic a first order phase transition (boiling), volume changes on rixing, and the complete phase behaviour of pure and mixed flids. Our formulation is based on a simple hole theory of theliquid state.

The total gange in the Gibbs free energy that is associated with the creation of  $N_0$  holes in a liquid of  $N_1$  molecules of size

$$\Delta G = \left[ v_1 N_0 E_h + P N_0 v_h + R T [N_0 \ln v_0 + N_1 \ln v_1] \right]$$
 (1a)

or, in reduced variables, by

$$\Delta G/rN_1E_h = G - G^0$$
=  $(1-1/v) + P(v-1) + T[(v-1)\ln(1-1/v) + (1/r)\ln(1/v)$  (1b)

where, r = hard core molecular volume/hole volume,  $v_h$ ;  $v_o = 1 - v_1 = N_o/(N_o + rN_1)$  = volume fraction of holes;  $\varepsilon$  = intermolecular interaction (contact) energy;  $E_h = \gamma \epsilon/2 = \text{hole}$  energy;  $\gamma = \text{lattice}$  coordination number;  $P = P \nu_h / E_h = \frac{1}{2} (E_h + E_h)$ reduced pressure;  $T = RT/E_h = \text{reduced temperature}$ ; V = macroscopic volume;  $V_o = rN_1v_h = \text{hard core volume}$  of molecules;  $\mathbf{v} = V/V_0 = 1/v_1 = \text{reduced volume}$ ;  $\mathbf{G}^0 = -1$ +P = reduced free energy of liquid without holes.

Equation (1) can be obtained directly from a lattice model in which the partition function is evaluated in the mean field approximation. It can also be obtained without appealing to a lattice description of the liquid state1.

The equilibrium number of holes can be determined by minimising the free energy with respect to  $N_o$ , or equivalently, the reduced volume v. Thus:

$$(\partial \mathbf{G}/\partial \mathbf{v})_{\mathbf{T}, \mathbf{P}, N_1} = 1/\mathbf{v}^2 + \mathbf{P} + \mathbf{T}[\ln(1-1/\mathbf{v}) + (1-1/r)(1/\mathbf{v})] = 0$$
(2a)

or

$$\frac{1/\mathbf{v} = f(1/\mathbf{v})}{= 1 - \exp[-1/(\mathbf{T}\mathbf{v}^2) - (1 - 1/r)(1/\mathbf{v}) - \mathbf{P}/\mathbf{T}]}$$
(2b)

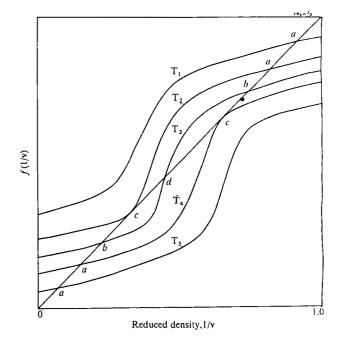


Fig. 1 Schematic representation of the graphical solution of the equation of state, equation (2b), below the critical point. The pressure is held constant and the temperature is varied. The various solutions to the equation of state produce the following kinds of behaviour in the Gibbs free energy, G: points a, absolute minima; points b, relative minima; points c, inflection points; points d, relative maxima.  $T_1 < T_2 ... < T_5$ .

The equation of state (2a or b) coupled with equation (1) defines the Gibbs potential in terms of its natural variables, T and P. Thus, all thermodynamic properties of the model are determined. Note that equation (2) is to be interpreted as defining the volume v in terms of T and P. This is an important distinction which assures that G will always be defined in terms of its canonical variables T and P; this further ensures that thermodynamic oddities, such as negative pressures, or compressibilities, will be avoided.

Equation (2b) can be solved graphically (Fig. 1). The pressure is held constant and the temperature is varied. At the lowest temperature, T1, only one solution to equation (2) exists (point a) and it corresponds to a high density phase (liquid). Point a corresponds to an absolute minimum in G. As the temperature is increased to T2, a second solution appears at the tangent, point c, which corresponds to a low density phase (gas). A corresponding inflection point occurs in G at point c whereas an absolute minimum in G still occurs at the new point a. At a temperature T<sub>3</sub> slightly above T<sub>2</sub>, there are three solutions to equation (2): two of them (points b) correspond to relative minima in G and the third (point d) yields a relative maximum in G. Thus, at temperatures near T3 both liquid and gas phases are possible and, in general, one phase will be metastable with respect to the other. At T4 the last vestige of a liquid phase is seen (metastable limit for liquid phase), and by  $T_5$  only a low density gas phase is present. There is a unique temperature, near T3 at which the free energy minima will be equal; this is the equilibrium saturation temperature. In the pressure-volume plane the locus of all such points define the classical saturation curve (binodal) (Fig. 2), and the locus of all the tangent points c defines a metastability limit (spinodal) (Fig. 2).

The spinodal is determined from  $\partial f(1/v)/\partial (1/v) = 1$  and equation (2) which defines the tangent points c (Fig. 1), and

$$P_{s} = -\frac{2v_{s}(v_{s}-1) \ln(1-1/v_{s}) + (1-1/r)(v_{s}-1) + v_{s}}{v_{s}^{2}[v_{s}-(1-1/r)(v_{s}-1)]}$$
(3)

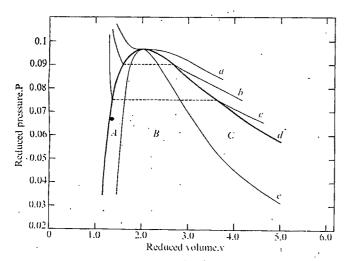


Fig. 2 Theoretical Pv diagram of a fluid for which the ratio of the hard core volume of the molecule to the hole volume is unity (r = 1). Along the equilibrium saturation curve (binodal), the Gibbs free energy, G of the liquid and gas phases are equal. The metastability limit (spinodal) is determined by equation (3). The set of all P, v points in the unstable region B yield relative maxima in G; G is nonanalytical (essential singularity) at the critical point. Tie lines connect isothermal states on the binodal, but not on the spinodal; spinodal temperatures are determined by equation (4). a, T = 0.500; b, T = 0.483; c, T = 0.455; d, equilibrium saturation curve; e, metastability limit. A, Metasstable liquid; B, unstable; C, metastable vapour.

for  $\mathbf{v}_s^0 \le \mathbf{v}_s < \infty$ ; where,  $\mathbf{v}_s$  and  $\mathbf{P}_s$  are the reduced volume and pressure that define the spinodal in the Pv plane (see Fig. 2), and  $\mathbf{v}_s^0$  is the value of  $\mathbf{v}_s$  for which  $\mathbf{P}_s = 0$ . The reduced spinodal temperature, Ts, is given by

$$T_s = [2(1-1/v_s)]/[v_s - (1-1/r)(v_s - 1)]$$
 (4)

The critical point not only satisfies equation (3), but also  $\partial^2 f(1/\mathbf{v})/\partial(1/\mathbf{v})^2 = 0$ . These conditions on  $f(1/\mathbf{v})$  are equivalent to the usual conditions for a critical point,  $(P_c, v_c, T_c)$ , that is,  $(\partial P/\partial V)_T = 0$ , and  $(\partial^2 P/\partial V^2)_T = 0$ , and they yield:

$$\mathbf{v}_c = 1 + \sqrt{r}; \ \mathbf{T}_c = 2r/(1 + \sqrt{r})^2$$
 (5)

$$\mathbf{P}_c = [2/(1+\sqrt{r})^2][r \ln(1+1/\sqrt{r}) + 1/2 - \sqrt{r}]$$
 (6)

Close examination of the equation of state shows that the free energy has an essential singularity at the critical point2.

The molar critical compressibility factor,  $Z_c$ , is given by

$$Z_{c} \equiv P_{c}V_{c}/RT_{c}$$

$$\equiv r(P_{c}v_{c}/T_{c})$$

$$= (1+\sqrt{r})[r\ln(1+1/\sqrt{r}) + 1/2-\sqrt{r}]$$
(7)

For  $r = 2, Z_c = 0.375$  which is identical to that obtained from the VDW and Berthelot equation of states3. The similarity of equation (2) and the VDW equation becomes more apparent when the former is expanded in virial form:

$$PV/RT \equiv r(Pv/T)$$
= 1+r(1/2-1/T)(1/v)+(r/3)(1/v)<sup>2</sup>
+(r/4)(1/v)<sup>3</sup>+...
(8)

The temperature dependence of the second virial coefficient is identical to that of the VDW equation.

Other observations are noteworthy. First, it is often assumed in hole theories that the total hole energy, E, is proportional to the number of holes  $(E = N_o E_b)$ . This is a valid approximation only at high densities. At low densities, hole-hole contacts become prevalent; when the random mixing correction for hole-

hole contacts is made, theenergy becomes  $v_1 N_0 E_h^{(l)}$  inificantly, if the v<sub>1</sub> factor is ignored, no phase transition will or. Second, the equilibrium saturation curve can be determd without appealing to the Maxwell construction or any otl construction outside thermodynamics. Third, the equate of state, equation (2a), implies a generalised correspoing states theorem; that is, all molecules of size r obey the se reduced equation of state. Fourth, for a homologous ser of liquids (such as the normal alkanes) the theoretical lling point increases with increasing molecular weight, observed experimentally.

The generalisation of equation (1) that is appririate for a binary mixture is straightforward. Formally, t system is ternary (two types of molecules, of sizes  $r_1$  and  $r_2$  lus holes). The only new parameter that is required to describbe mixture is the interaction energy  $\varepsilon_{12}$  between the two comments. The free energy of mixing,  $\Delta G_m$ , is given by

$$\Delta G_m = \overline{r}N \left(\overline{E}_h \mathbf{G}_{12} - x_l E_h^{1} \mathbf{G}_1 - x_2 E_h^{2} \mathbf{0}\right)$$
 (9)

and

$$G_{12} = -1/v + Pv + T[(v-1)\ln(1-1/v) + (1/r)\ln(1/+ + (x_1/r_1)\ln x_1 + (x_2/r_2)\ln x_2]$$
(10)

where,  $N = N_1 + N_2$ ;  $\bar{r} = (r_1 N_1 + r_2 N_2)/N$ ;  $z = 1 - x_2 = r_1 N_1/\bar{r}N$ ;  $E_h^1 = \gamma \varepsilon_{11}/2$ ;  $E_h^2 = \gamma \varepsilon_{22}/2$ ;  $\bar{E}_h = \gamma/2(x_1^2 \varepsilon_{11} + 2x_1 x_2 \varepsilon_{12} + x_2^2 \varepsilon_{22})$ ;  $T = RT/\bar{E}_h$ ;  $P = Pv_h \bar{E}_h$ ;  $v = V/V_o$ ;  $V_o$  $= \bar{r}Nv_h$ ; and  $G_1$  and  $G_2$  are the free energies or the pure components, 1 and 2 [compare with equatic (1b)]. The enthalpy, entropy, and volume changes that oca on mixing are obtained in the usual way by taking derivates on  $\Delta G_m$ . The functional form of the equation of state of the ixture is the same as that for the pure fluid, except that the reded variables are as in equation (10), and r is replaced by  $\vec{r}$ . Thextension of the theory to any number of components onlyrequires the obvious generalisation of equations (9) and (10) Only in the limit  $\mathbf{v} = \mathbf{v}_1 = \mathbf{v}_2 = 1$  does  $\Delta G_m$  reduce to the Fory-Huggins expression. In the general case of zero volumechange, v =  $x_1v_1+x_2v_2$ , there are still significant contribution of  $v_1$  and  $v_2$ to both the entropy and enthalpy of mixing.

The chemical potential  $\Delta \mu_1$  is given by:

$$\Delta\mu_{1} \equiv (\partial \Delta G_{m}/\partial N_{1})_{P,T,N_{2}} = r_{1}(\bar{E}_{h}G_{12} - E_{h}^{1}G_{1} + x_{2}\partial(\bar{E}_{h}G_{12})/\partial x_{1})$$
(11)

and  $\Delta\mu_2$  can be obtained by symmetry.

For liquid-liquid equilibria, the binodal is letermined by equating chemical potentials across the liquid hases. For a non-polar polymer mixture  $(r_2 \gg r_1)$  where  $\epsilon_1 = (\epsilon_{11}\epsilon_{22})^{1/2}$ both upper and lower critical temperatures of solution are predicted. Theoretical, negative, excess volumes are also obtained under these conditions. Equating chemical potentials across liquid and vapour phases allows for he complete determination of the boiling point composition diagram (distillation diagram). For  $r_1 = r_2 = 1$  and  $\varepsilon_{12} = (\varepsilon_{11}\varepsilon_{22})^{1/2}$ , nearly ideal behaviour is obtained. Deviations o  $\epsilon_{12}$  from the geometric mean, or large dissimilarities in molecular size, cause maxima or minima to occur in these diagrans; azeotropes are thus predicted. To our knowledge, no other molecular theory has been presented which predicts the entire range of distillation phenomena from a unified point of view.

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## Load-being structures and crysl intergrowth

As far as 1m aware, no one has attempted to place the concept of cstal-crystal intergrowth on a quantitative basis. The object this note is to calculate the probability of the formation ca three-dimensional load-bearing structure on the basis of ystal-crystal intergrowth.

The originf the load-bearing capacities of structures like inorganic ceents and bone is still disputed. In each of these structures, temselves composites, the solid component, generally asimed to be the main load-bearing element, is mainly crystline in nature, though usually of submicrometre size. Two mehanisms have been proposed to explain load-bearing capaties. In the first it is assumed that the solid particles fon a physically bonded structure<sup>1-3</sup> whereas the other assmes that the solid particles form a three-dimensional structure by crystal-crystal intergrowth at the points of contact<sup>4-7</sup>. I either case, the points of contact or the points of intergrowt must be numerous and spread throughout the volume for to structure to be functional.

In inorgan cements as well as in bone, crystallisation begins in isolated agions. Subsequent crystallisation and crystal growth bringthe crystals from different regions into contact. Thus, when vo crystals from two different regions meet, they do so at a radom orientation. The case of apatite crystals in long bones i somewhat complicated. The apatite crystals in long bones o a newly born baby are randomly oriented; those in mature boe, however, have a preferred orientation with the c axis paralle to the long axis of the bone.

When two crystals from two different regions come into contact two possibilities may arise. In the first case, the atoms at the interfece can be considered to belong to the crystal structures of both crystals and because of the structural continuity the intercrystalline bond is nearly as strong as the intracrystalline bond. In the second case there is no such relationship and the intercrystalline bond is much weaker than the intracrystalline bond so the crytsals can be considered to be physically bonded.

For ideal cystals, crystal-crystal intergrowth can only occur when the crystals meet each other in a symmetry related indistinguishable orientation. For micrometre sized real crystals some angular relaxation of the order of the mosaic spread in a single crystal—that is 3-4°—is expected. The probability that two crystals will meet each other in this orientation, by accident, can be calculated.

Let (hkl) be the crystal plane across which the crystal-crystal intergrowth is going to occur. Let c be an axis normal to the plane having an n degree rotation symmetry and a be an axis in the plane. If a second crystal is brought into contact with the first an intergrowth will occur when both c and a axes of the two crystals are within the angular relaxation zones of each other. Statistically, the second c axis can approach the first from any point on a hemisphere drawn round the first. The probability of two c axes being within the relaxation cones of each other is given by

$$P_c = (1 - \cos 4^\circ) = 0.002$$

The a axis will repeat n times in 360°, so that when two c axes are parallel to each other the probability of the two a axes coming within each other's relaxation zones is given by

$$P_a = n \times 4^\circ/360^\circ = n/90$$

so that the total probability of intergrowth is

$$P_t = P_c P_a = n/45,000$$

The probability of a third crystal joining either of the first two in intergrowth orientation is given by  $P_t^2$  and so on. Thus the probability of the formation of an extended structure on this basis is vanishingly small. Even if most of the c axes are parallel to each other, somewhat like apatite crystals in mature bone, the probability that a load bearing structure will form will still be extremely small. The probability of obtaining an aggregate 1 mm long from micrometre-sized crystals on this basis will be  $(n/90)^{899}$  where n is a small integer. This very low probability is consistent with a scanning electron microscopic work on plaster of Paris blocks. This work did not reveal any definite evidence for the occurrence of extensive crystal-crystal intergrowth though one of the objects of the study was to search for it.

In the case of physical bonding of the crystals there is no requirement of any continuity of atomic arrangement between the participating crystals, so bonding will occur whenever crystals come sufficiently close to each other. The probability of the formation of an extensive structure on this basis depends only on the packing density of the crystals and on their size and shape<sup>10</sup>.

Thus the most probable mechanism of the origin of loadbearing capacities of bone and inorganic cements is the physical bonding of the crystals.

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# Direct observation of alkali metal colloids in alkali halide crystals

SIEDENTOPF¹ observed that sodium chloride crystals containing atomically dispersed F centres turned blue and exhibited Tyndall scattering when annealed at 400° C. These effects were attributed to colloidal particles of sodium metal formed in NaCl crystals by aggregation of F centres during the annealing process. Savostianova² applied the Mie³ theory to absorption and scattering of light from colloidal metallic particles suspended in dielectric media and predicted colloid sizes in excess of 80 nm. The blue colour of natural rocksalt, investigated⁴ using optical absorption and photoconductivity, was similarly attributed to colloids. Ionising radiation also produces colloidal absorption bands in irradiated alkali halide crystals under certain conditions⁵-10.

Increasingly sophisticated techniques have been applied to the study of these colloidal centres, for example, ultramicroscopy<sup>11</sup>, diffuse X-ray scattering<sup>12,13</sup>, NMR<sup>14,15</sup>, EPR<sup>16-18</sup>, electrical conductivity<sup>19</sup> and surface replication<sup>20-26</sup>. Despite 70 years of investigation, their existence has still remained controversial, partly for the want of a direct method for observing actual colloidal particles *in situ*, and in part to disagreement over possible mechanisms of colloid formation<sup>27-29</sup>, particularly over the role of existing defects such as dislocations and impurities<sup>11,25,30-32</sup>.



Fig. 1 a, Spherical inclusions preferentially nucleated along dislocation lines in KCl additively coloured with potassium to  $10^{25}$  F m<sup>-3</sup> and annealed for 1 h at 673 K to produce a colloid absorption band. b, Cylindrical inclusions along a dislocation line in KI additively coloured with potassium to  $8 \times 10^{23}$  F m<sup>-3</sup> and annealed for 4 h at 433 K. c, Faceted inclusion in KI additively coloured with potassium, showing weak Moiré fringes normal to the diffraction vector g.

Transmission electron microscopy is a sufficiently direct technique but attempts<sup>5</sup> to observe existing lattice defects in alkali halide crystals using this technique<sup>33</sup> have always been hampered by the catastrophic radiation damage induced by the investigating electron beam. New techniques<sup>24,35</sup> involving electron microscopy at liquid helium temperature can circumvent this problem and allow almost rousine electron microscopy of existing lattice defects; these methods have been applied recently to studies of dislocations in deformed crystals<sup>36</sup> and interstitial stabilisation in irradiated crystals<sup>37</sup>. We have here applied them to the first direct electron microscopical observation of what we believe are alkali metal colloids in additively coloured alkali halide crystals.

Potassium chloride crystals (Harshaw Chemical Company) were additively coloured with potassium vapour, using van

Doorn's method38, to produce an F-centre densityquivalent to a stoichiometric excess of potassium ator between  $2\times10^{24}$  and  $10^{25}\,\mathrm{F}\,\mathrm{m}^{-3}$ . The crystals were section parallel to {100} and {110}, annealed in dry nitrogen vacuum for 1 h at 673 K and either rapidly quenched or fuce-cooled. Optical absorption measurements indicated a largbsorption band peaking between 750 and 850 nm, corronding to average colloid diameters in the range 90-120 nnn the Mie theory. Potassium iodide crystals (Quartz et lice) were similarly coloured to an F-centre density of 8×23 F m<sup>-3</sup>, sectioned and annealed for 4 h at 433 K. Opticabsorption measurements indicated a broad colloid abstion band peaking between 700 and 900 nm. Thin foils we prepared from annealed material using a precision chemi polishing method39 and examined at liquid helium temperare34,36 in a 100-kV electron microscope. Approximately 15 s observation time was available for image recording in KCl undan incident electron beam intensity of ·18 A m<sup>-2</sup> before aggration of interstitial radiation defects impeded observation<sup>17</sup>; approximately 45 s was available in KI at 45 A m<sup>-2</sup>.

We observed images from inclusions (Fig. 1a) which we ascribe to potassium metal colloids. The obserd inclusion dimensions vary between 50 nm and > 400 nmhose of the latter size being of the order of the foil thicknessVhen small, these inclusions are approximately spherical; larr inclusions are often faceted (Fig. 1c). They were observeoy structure factor contrast40,41 appropriate to coherent prepitates with different extinction distances  $\xi_g$  from the matri ( $\xi_{220}$  (KCl) = 87 nm;  $\xi_{220}$  (KI) = 70 nm;  $\xi_{220}$  (K, f.c.c.= 170 nm). Absorption contrast was also observed. The dtribution of inclusions was found to be microscopically inhorgeneous, the average density being of the order 1017 m-3 Independent measurements using a surface replication techniue26 confirm this size distribution, inhomogeneity and morhology. The number of potassium atoms which could be stabised in aggregates of this size and distribution adequately acounts for the measured loss of F centres during anneal.

Absence of any additional diffraction feature in selected area diffraction patterns from regions containin these inclusions suggests that potassium atoms are retained itheir original face-centred cubic (f.c.c.) positions within thealkali halide matrix, with a lattice parameter not too different frm the matrix  $(a_0 \text{ (KCl)} = 0.629 \text{ nm}; a_0 \text{ (KI)} = 0.707 \text{ nm}; a(\text{K, f.c.c.}) =$ 0.655 nm;  $a_0$  (K, b.c.c.) = 0.535 nm at 3001). When the inclusions were sufficiently large, weak Moiréfringes were observed (Fig. 1c) similar to those observed 42 for misfitting coherent precipitates. The fringe spacing is not wil defined, but nevertheless corresponds to a coherency strain<sup>43</sup>magnitude of the order 0.5% in both KCl:K and KI:K. The actal differences in alkali metal and alkali halide lattice parametes correspond to expected coherency strains of 0.31% for KCIK (matrix in compression) and -0.76% for KI:K at 300 K (matrix in tension), using for the bulk modulus of f.c.c. octassium the value (4 GN m<sup>-2</sup>) available for body-centred cubic (b.c.c.) potassium metal. The smallness of the strain values arises from the large compressibility (small bulk modulus) of the alkali metal lattice compared with the stiffness of the alkali halide matrix. Little matrix strain-field contrast44,45 is observed surrounding the larger inclusions as expected from he strain field visibility limits established by Ashby and Brown<sup>44</sup>.

In crystals which were rapidly quenched from the annealing temperature, inclusions were often surrounded by dislocation tangles. When care was taken to cool crystals sowly from the annealing temperature to preserve the existing dislocation structure, inclusions were observed to have preferentially nucleated along existing dislocation lines (Fig 1a and b). In such cases, they were more uniform in size and were regularly spaced along the dislocation lines; their shape vas distorted in the direction of the dislocation line. This observation confirms the special role of dislocations in sensitising the nucleation of colloids. We have so far also made observations on similar inclusions in the systems KBr:K and NaCl:Na.

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#### The aerodynamic diameter of branched chain-like aggregates

AGGREGATES of a large number of submicron primary particles are produced by industry and automobiles. Such aggregates are also used in laboratory studies, so the fluid drag on these aggregates is of considerable interest.

Vomela and Whitby have studied the fluid-drag on chain-like aggregates, produced by an exploding wire as well as by hydrocarbon flames. Their data show a Stoke's diameter (diameter of a sphere having the same bulk density and settling rate as the aggregate) larger than the volume equivalent diameter (diameter of a sphere having the same volume as the aggregate) of an aggregate, which obviously cannot be correct4.

We deal here with aggregates of submicron iron oxide particles, produced by an exploding wire in an aerosol tank. The aerosol is labelled with 59Fe by pre-irradiation of the wire with neutrons. The primary particle size distribution can be varied by varying the specific explosion energy.

Some time after the explosion, when aggregates are formed due to coagulation, the aerosol is sampled and analysed with a centrifugal spectrometer according to Stöber and Flachsbart<sup>2</sup>, which deposits the aerosol particles on a strip according to their aerodynamic diameter (diameter of a unit density sphere having the same settling rate as the aerosol particle), which is characteristic for the fluid drag.

The relationship between the mass and the aerodynamic diameter of the aggregates can be obtained from measurements of the radioactivity and particle number (obtained by counting from optical and for small aggregates from electron microscope pictures) along the deposition strip. The primary particle size distribution is determined using electron microscopy, with negatively stained catalase crystals3 as an internal length standard. It has been shown that the primary particle size distribution can be approximated by a log normal size distribution (characteristic features: the geometric mean diameter  $d_{1s}$ and the geometric standard deviation  $\sigma_s$ ). The number n of primary particles of the aggregate can be calculated from the equation:

$$m = (\pi/6) \rho n d_{1g}^{3} \exp(4.5 \ln^{2} \sigma_{g})$$
 (1)

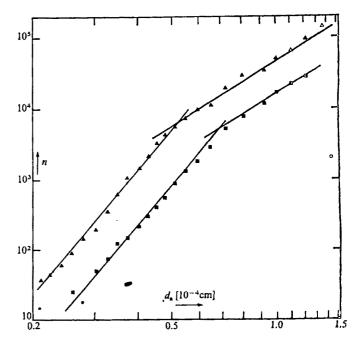


Fig. 1 The relationship between the number (n) of primary particles and the aerodynamic diameter  $(d_a)$  of branched chain-like aggregates of submicron iron oxide particles.  $\blacktriangle$ ,  $d_{1g} = 0.027 \ \mu m$ ,  $\sigma_g = 1.8$ ;  $\blacksquare$ ,  $d_{1g} = 0.041 \ \mu m$ ,  $\sigma_g = 1.8$ .

with: m = mass of the aggregate and  $\rho = \text{density}$  of the aerosol material = 5.24 g cm<sup>-3</sup> for the particles studied by us (aerosol material is Fe<sub>2</sub>O<sub>3</sub> as could be determined by means of X-ray diffraction).

With the aid of equation (1) the relationship between the number of primary particles and the aerodynamic  $d_a$  of the aggregates can be obtained from the measurements of the aerosol mass and number distributions on the deposition strip in the aerosol centrifuge.

Figure 1 shows those experimental results already obtained for two different primary particle size distributions. For  $n \le 5 \times 10^3$ ,  $d_a$  is proportional to  $n^{1/6}$ , whereas for  $n \le 5 \times 10^3$   $d_a$  is proportional to  $n^{1/3}$ .

Figure 2 shows that the configuration of aggregates with less than about  $5 \times 10^3$  primary particles is linear in contrast to

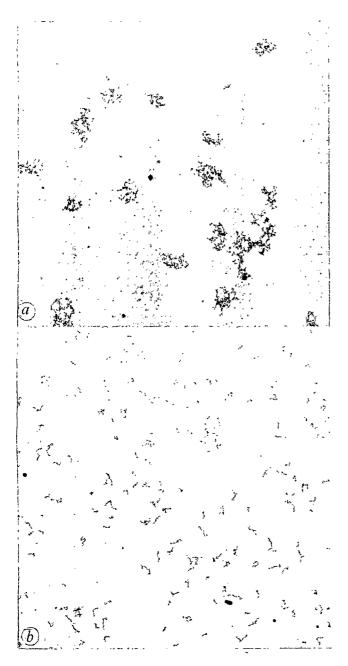


Fig. 2 Electron microscope pictures of branched chain-like aggregates of submicron iron oxide particles ( $d_{1g} = 0.027$ ,  $\sigma_g = 1.8$ ). a,  $d_a = 0.925 \mu m$ ,  $n = 3.10^4 (\times 165)$ ; b,  $d_a = 0.259 \mu m$ ,  $n = 100 (\times 700)$ .

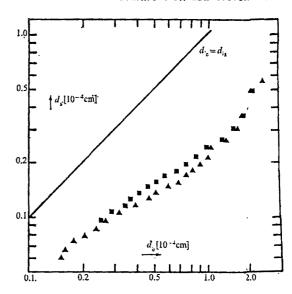


Fig. 3 Relationship between the volume equivalentameter  $(d_e)$  and the Stoke's diameter  $(d_s)$  of branched chain-li aggregates of submicron iron oxide particles.  $\triangle$ ,  $1 d_{1g} = 0.027a$ ,  $\sigma_g = 1.8$ ;  $\blacksquare$ ,  $1 d_{1g} = 0.041 \ \mu m$ ,  $\sigma_g = 1.8$ .

aggregates with more than about  $5 \times 10^3$  prinry particles, which are irregular three-dimensional networks.

In accordance with Stöber<sup>4</sup>, the following relaonship can be derived for the linear aggregates of polydiserse primary particles

$$d_n = k n^{1/6} \sqrt{(\rho/\rho_0)} d_{1s} \exp(2ln^2\sigma_s)$$
 (2)

with k = proportionality constant,  $\rho_0 = 1 \text{ g cn}^{-3}$ .

The values of k resulting from our experiment (k = 0.92 and 0.87 respectively) are somewhat smaller than itöber's result (k = 1.077) for linear aggregates (up to eight prinary particles) of monodisperse polystyrene spheres.

The relationship between the number of primay particles and the aerodynamic diameter of the aggregates with more than  $5 \times 10^3$  primary iron oxide particles, has been found to be

$$d_a = f n^{1/3} \sqrt{(\rho/\rho_0)} d_{1g} \exp(1.5i^2 \sigma_g)$$
 (3)

with: f = 0.903 for cluster aggregates as results from Stöber's study; f = 0.250 for branched chain-like aggregates with more than about  $5 \times 10^3$  primary particles, as results from this study.

The difference between the values of f found an be ascribed to the totally different packing densities of the aggregates studied by Stöber (cluster aggregates of monodsperse polystyrene spheres) and ourselves (fluffy aggregates of ubmicron iron oxide particles).

The relationship between the Stoke's diamete, which can be calculated<sup>4,5</sup> from  $d_a$  and the volume equivalent liameter of the aggregates, is shown in Fig. 3. In contrast to the lata of Vomela and Whitby<sup>1</sup> we found Stoke's diameters of the branched chain-like aggregates smaller than their volume equivalent diameters, which is in agreement with theoretica considerations.

The present results are of great importance, for example, in describing coagulation of solid aerosol particles. Until now the approximation of the droplet model was used in which the particles are assumed to be droplets which unite to form one new droplet. In fact, this implies that the Stcke's diameter of chain-like aggregates equals the volume equivalent diameter. Obviously, this is incorrect.

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#### Decline c PCB concentrations in North tlantic surface water

POLYCHLOROPHENYL (PCB) concentrations in North Atlantic surface waterhave declined fortyfold since 1972, following the cessation of ertain industrial uses of those compounds. Industrial sal of PCB for use in products which would allow leakage to thenvironment, for example in plastics, inks and paints were spped in the United States and Sweden during 1970-711; anin 1972-73 other European countries and Japan initiated a sinlar action<sup>2</sup>. In 1971 and 1972 average surface concentration of PCB in the open North Atlantic Ocean were about 30 ng<sup>-1</sup> (ref. 3 and C. E. Olney and J. J. Quinn, unpublished) 3y April of 1973 the concentration in the Sargasso Sea had been educed to 1 ng l-1 (ref. 4); five months later we found that the decline had levelled at 0.8 ng l<sup>-1</sup>, a concentration which was stl present in February 1974 in the north-west Atlantic. The ccumulated data are presented in Table 1. These observations evealing a reduction in surface water concentrations of PCB:ompare well with our February 1974 measurements of a terfold decrease of PCB in the atmosphere over the north-west Alantic since 1973 (ref. 5), and a fivefold decrease

Table 1 PCI concentrations in North Atlantic surface waters\* 1971-74

Date	Mean PCB (ng 1 ¬)	Sampling region	No. of samples	Analytical‡ method	Ref.
8/71	25	Iceland to Nova Scotia	8	Α	§
7/72	41	Newfoundland to			•
		Portugal	8	В	3
8/72	36 '	Portugal to			
		Norwegian Sea	11	С	3
9/72	30	Woods Hole to Bermud	la 27	С	3 3
4/73	1	Sargasso Sea	8	Α	4
9/73	2	Azores to 09°N, 40°W			
		to Barbados	10	С	3
9/73	0,8	Sargasso Sea to			
a		New York Bight	9	С	3
2/74	0.8	New England			
		Continental Shelf	6	С	3

\* 0-30 m.

† Blank values for PCB in methods A, B and C were 0.9, 0.5 and 0.3 ng  $1^{-1}$ , respectively. Replicate analyses using methods B or C showed an average variation of  $\pm$  20% at the 1 ng  $1^{-1}$  level. Quantitation methods are presented in refs 3, 5, 6.

‡ A, Extraction of 0.5-3.8 1 with chloroform or dichloromethane; B, extraction of 201 with 5% ether in hexane; C, passing 40-601 through Amberlite XAD-2 resin followed by elution with boiling acetonitrile

§ C. E. Olney and J. J. Quinn, unpublished information.

since 1972 in the PCB content of mixed plankton collected along 09°N latitude in September of 1973 (ref. 6).

The data require that about 2×104 tons of PCB were lost from the upper 200 m in less than one year3. To explain this loss, four processes must be seriously considered: (1) apparent dilution of dissolved PCB by vertical and horizontal advection and diffusion; (2) association of PCB with sedimenting particles; (3) evaporative codistillation into the atmosphere and (4) biological and chemical degradation.

The information obtained from the monitoring of bomb fallout radionuclides is relevant to the present problem because like PCB, they are delivered to the ocean from the atmosphere, can exist in dissolved and particulate phases, and experienced a similar decreased input after the nuclear test ban agreements of

Table 2 PCB concentrations in North Atlantic subsurface waters\*, 1973

		1775		
Date of collection and analyses†	n Pos Latitude	ition Longitude	Depth (m)	PCB (ng 1 <sup>-1</sup> )
19/9/73	09° 00′ N	40° 00′ W	10 100 200 400 500 700 1,000 2,000	4.3 2.0 1.4 1.1 1.3 1.5 1.0 2.1
2/10/73	32° 25′ N	70° 20′ W	3,000 10 100 300 600 900 5,100	1.0 0.6 0.8 0.9 0.5 1.9

<sup>\*</sup> Reproducibility at the 0.5 ng 1<sup>-1</sup> level was ± 40%. The blank was produced by recycling 401 of XAD-2 extracted seawater through the entire procedure and averaged 0.3 ng 1<sup>-1</sup>.

† Method C was used.

1963. For example, strontium-90 which is mainly in the dissolved state in seawater, required more than five years after the test ban treaty to decline to one-half the surface water concentrations of 1963 (ref. 7). Thus, depletion of dissolved PCB from the upper ocean by advection and diffusion seems too slow to explain the observed rate. The rate of PCB removal from the surface waters more closely resembles the behaviour of the plutonium nuclides 239-240Pu which are believed to be associated with particulates8. The vertical distribution of plutonium isotopes in the Atlantic was best explained as being associated with particles falling at rates of 70-392 m yr<sup>-1</sup>. The well known affinity of PCB for solid surfaces suggests that adsorption on falling particles could be a major transfer mechanism. In fact, if 2×104 tons or less of PCB has been transferred to the deep ocean each year (1970 was the peak production year) for the past 10-15 yr and was dispersed throughout the entire water column, the concentrations to 3,500 m would still be less than 2 ng l<sup>-1</sup>. Two 1973 profiles of PCB in the water column are presented in Table 2. The profile at 40° 00'W was typical of eight other stations in the North African and Cape Verde Basins; in contrast, the station at 70° 20'W revealing very low PCB levels at the surface, was similar to nine other stations in the North American Basin the same year.

We are, at present, unable to ascertain the relative importance of evaporative codistillation of PCB from the surface water or of biogeochemical degradation. Either process could explain the lower surface water concentrations in the North American Basin. There is, as yet, no evidence of biological metabolism of PCB in the marine environment.

We note the rapid response of the open-ocean mixed layer to the reduction of industrial discharges of PCB. Efforts to determine the mechanism of this efficient transfer process are

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#### Origin proposed for non-protein amino acids in meteorites

THE amino acids that have been identified in several meteorites1,2 can be divided into two classes: those present in proteins and those which are not. The presence of the non-protein amino acids, D- and L-β-aminoisobutyric acid and β-alanine, is described as evidence for the indigenous origin of meteorite amino acids and against terrestrial contamination<sup>1</sup>. It is implied that these \beta-amino acids arise by prebiotic condensation of ammonia, methane, hydrogen, water and other simple primordial molecules3. The β-amino acids and other amino acids can arise from simple molecules by Fischer-Tropsch synthesis in the laboratory. Hydrogen, carbon dioxide and ammonia were heated to high temperatures in the presence of natural catalysts expected to be present in the solar nebula4.

In other experiments, thermal synthesis of amino acids occurred in a simulated primitive atmosphere of methane, ammonia and water, followed by acid hydrolysis of the products. β-Alanine was obtained in high yield, relative to other amino acids, and the straight-chain compound, \beta-amino-n-butyric acid<sup>5</sup>. These amino acids were postulated to arise respectively from hydrolysis of β-aminopropionitrile and the nitrile intermediate arising from addition of ammonia to crotononitrile. Evidence for this route was the identification of succinic acid and N-methyl-β-alanine which are likely hydrolysis products of nitrile intermediates. Production of β-aminoisobutyric acid was not reported in these experiments.

β-Aminoisobutyric acid is a rare amino acid with a curious distribution in nature. It has been isolated only from human urine6, iris bulbs7, flatworms8 and edible mussels9. Man is probably the only mammalian species to excrete p-(-)β-aminoisobutyric acid10 though there is chromatographic evidence for its presence in rabbit urine11. Metabolic experiments suggest that the pyrimidine base thymine, from nucleic acid catabolism, is the precursor of β-aminoisobutyric acid in the human<sup>12-14</sup>. Similarly, metabolic production of  $\beta$ -alanine in rat liver is from the pyrimidine uracil<sup>15</sup>.

By analogy with these known reaction sequences catalysed biologically, I suggest that heterocyclic compounds could also act as precursors for the \beta-amino acids found in meteorites. In this case, scission of the ring of heterocyclic compounds formed prebiotically would occur under the catalytic conditions of extremes of temperature and radiation thought to occur in space.

The purines, adenine and guanine, and a substanesembling uracil have been detected in the Orgueil meteoriteXanthine, thymine, uracil and derivatives have also been objed experimentally by mimicking supposed prebiotic condits4.

This chemical route to certain non-protein ino acids described above would presumably give racemicixtures of both stereoisomers, for example, both D-, ant-β-aminoisobutyric acid, as found in meteorites1. It would interesting to know if thymine, dihydrothymine, uracil andated compounds, when heated under supposed prebiotic clitions, did give traces of the β-amino acids.

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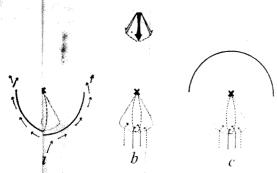
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#### Evidence for downwind flights by host-seeking mosquitoes

THE behaviour of flying insects which make up of airborne chemicals in their search for food, oviposition ites or sexual partners can be separated into two phases, th search flight bringing them into contact with the attractant lume and the approach flight leading them to its source1,2. Thesecond phase is characterised by upwind orientation in reponse to the appropriate chemical stimuli3-6. Mosquitoes anknown to fly upwind as they approach a warm-blooded hos -9. For most insects relatively little is known of the pattern othe preceding search flight which Haskell2 described as 'wandering', but where the wind is light it may be upwind1. Experiments n West Africa have shown that the majority of mosquitoes enteed flight traps from the downwind side, that is they appeared to have been flying upwind, regardless of the presence or absence of a host (ref. 10 and W. F. Snow, unpublished). Iere we report experiments that unexpectedly provide evidence br the opposite type of behaviour.

If the search flight is in a generally upwind direction, it should be possible to divert mosquitoes round ahost by setting up a barrier on the downwind side and so provile a measure of protection from their attacks. The barrier would need to be sufficiently far away for the mosquitoes to encounter it before they detected the presence of the host, so that directed responses towards the host would not be elicited. This distance was estimated by Gillies and Wilkes<sup>11</sup> to be less than 18 m for a host the size of a man. A host stationed at this distance upwind



Teffect of barriers on mosquitoes flying upwind towards a st. Crosses represent hosts protected by, a, semicircular bærs on the downwind side; c, upwind side; and, b, unproted. The thick arrow represents wind direction, and the fine aws the flight paths of mosquitoes. The plume of attractant in the host is shown as an ellipse, swinging in a narrow arc in a variable wind.

of the barrishould, therefore, receive fewer bites than a bait in the open one protected on the upwind side (Fig. 1).

A bait staned downwind of the barrier (Fig. 1 c) might well have in within its wind shadow which could have affected the insity of insects on the leeside regardless of their direction of ght12. So we decided to compare the attack rate on a host upind of a barrier with that on an unprotected bait at the pointmarked X in Fig. 1a and b. A semicircular fence of plastic mquito netting was constructed with the opening perpendiculato the prevailing wind. The fence was 2.9 m high, 57 m long ar formed part of a circle of radius 18.3 m. It was set up in arextensive area of cleared farmland near Sapu, The Gambia0.5-1 km from the nearest village. Two human 'baits' sat 36n apart at points a and b (Fig. 1) and caught the mosquitoes i tubes as they settled to bite, changing places at halftime. Wil speed was measured at a height of 1 m and wind direction wasecorded at 1-2 min intervals. Weather conditions were favourale on eight nights in July 1973, for a total catching time f 6.5 h. On these nights the wind was within 45° of the prevaing direction for 96% of observations. Mean wind speed was 2 ns<sup>-1</sup>. The fence was highly permeable, air flow at a point 3 m do nwind from it being reduced by a factor of 0.62 at ambient winespeeds.

In a seconseries of experiments, the half-circle of fence was converted in a complete circle of the same height. Paired catches by hman bait were done in the same way as before, except that the control bait was 70 m from the nearest part of the fence. Mean wind speed in the open was 0.9 m s<sup>-1</sup>.

The results with the half-circle fence showed that the mean nightly ratio of mosquitoes caught upwind of the fence to those in the open was 0.97. Thus virtually identical numbers were caught by the 'protected' and by the exposed bait (total catch 814 mosquites, 84% being Mansonia (Mansonioides) spp.). On the other hand, the results of 12 paired catches inside and outside the complete circular fence showed that the mean nightly ratio if catches inside to catches in the open was 0.69.

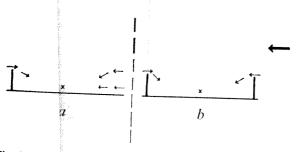


Fig. 2 Section through, a, semicircular fence and, b, circular fence to show possible approach paths of mosquitoes (fine arrows) to the host (X). Wind direction is indicated by the thick arrow on the right.

The difference for catches of Mansonia spp., which totalled 2,048 mosquitoes, was highly significant (P < 0.01). This shows that the circular fence reduced the numbers reaching the bait by something like 30%.

With the half-circle fence mosquitoes could have reached the bait by flying downwind at any level or upwind only over the top of the fence (Fig. 2a). The circular fence would have excluded low-level fliers and only permitted the approach of high-flying insects (Fig. 2b). If the mosquitoes that reached the bait in the first experiment had mainly approached by flying over the fence, the exclusion of low-flying insects in the second experiment would not have had any effect on the catch. As, on the contrary, there was a marked reduction in this catch, we conclude that a significant number of mosquitoes reached the vicinity of the bait in the first experiment by flying at a low level downwind. Their search flight before the detection of host stimuli must therefore have been in a generally downwind direction and low enough for them to encounter the odour plume from a ground level host. Thereupon they presumably turned and tracked back upwind toward the host. This would make for a much more efficient host-seeking strategy since, for a given output of energy, the insect would be able to cover a much larger area than if it were restricted to upwind movements.

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#### Diffuse competition in Lepidoptera

THE effects of interspecific competitive encounters have traditionally been viewed in terms of species replacement, but recent workers1.2 have recognised the importance of weak or diffuse competition, which leads only to reduced niche volume. Evidence of such niche restriction has been obtained from the study of bird communities, where it has been shown that species often expand niche dimensions in areas with few species<sup>2,3</sup>. For example, one-third as many bird species are present on the island of Puercos as on the nearby Panamanian mainland and the insular birds forage in a wider range of habitat types than conspecific mainland birds4. Unfortunately this rather direct method of relating changes in niche width to changes in competitive background is often difficult to apply but the importance of competition can be evaluated in other ways. It is generally conceded that if competition occurs at all, it will be strongest among phenotypically similar species.

More explicitly, the intensity of competition which a species faces should be inversely related to its phenotypic isolation, where such isolation is a complex function of the phenotypic distance between a species and coexisting species which share limiting factors.

The relationship between intensity of competition and species abundance is not likely to be a simple one. Much depends on the limiting factors involved in the competition. The abundance of resource-limited species is, on the short run at least, related to the abundance of the resource. The data from Puercos<sup>4</sup> do, however, indicate a flexibility in resource abundance, depending on the utiliser's definition of the resource. The potential overlap of such definitions will clearly lead to competition. In cases where resource limitation is unimportant, the effects of competition on abundance could be equally important, but not necessarily any more clear-cut; few limiting factors are totally nonspecific, yet, equally few are totally specific.

It is difficult to make a general case for the resource limitation of herbivorous insect populations. Certainly, as Hairston, Smith and Slobodkin<sup>6</sup> point out, insects rarely strip their food plants of foliage. In non-resource limited species the possibility of competition arises when several species share mortality factors<sup>7</sup> such as parasites, predators or disease, for a group of species will then tend to be regulated as a whole rather than as individual units.

We decided to analyse patterns of abundance in lepidopterans because large numbers of species are present in a local fauna. Moreover, the families to which these species belong are represented by differing numbers of species. Species which belong to small families will be more phenotypically isolated from the lepidopteran community as a whole than species belonging to families represented by many species and the present study demonstrates that species in these small families tend to have higher population densities.

As it is difficult to sample the abundance of an entire lepidopteran community in any meaningful way, this study has been restricted to an analysis of the abundance of night flying moths. To reduce the size variation of the species considered and to avoid taxonomic difficulties, Microlepidoptera were

Table 1 Family differences in species abundance

	Si	te 1*	Si	te 2†
	No. of species	No. of individuals/	No. of species	No. of individuals/
Noctuidae Geometridae	401	species 68.61	307	species 21.36
Notodontidae	168 43	91.51 156.02	140 43	27.79 72.42
Arctiidae Sphingidae	34 22	201.35 130.91	28 21	50.54 42.90
Ten small famil	lies 32	223.84	32	70.84

One 'sample' is a collection from one light over one season.
\*Perth Road, Frontenac Co., Ontario (1970-1971); four samples, total no. of individuals = 66,484.

†Chaffeys Locks, Leeds Co., Ontario (1969-1970); four samples, total no. of individuals = 18,146.

excluded from study. Collections were carried out at two sites approximately 10 miles apart. Site 1 was situated in a large area of undisturbed deciduous forest, while site 2 was situated in an area which had been subject to limited clearing. Moths were collected with a 20 W ultraviolet light set against a white cloth background. Trapping periods have been listed elsewhere<sup>8</sup>, but generally extended from May until mid September.

Fifteen families of moths were represented in the collections. At site 1, 401 species of Noctuidae, 168 species of Geometridae, 43 species of Notodontidae, 34 species of Arctiidae and 22 species of Sphingidae were collected. Ten additional families (Citheroniidae, Drepaniidae, Epiplemidae, Euchromiidae, Lasiocampiidae, Lymantriidae, Nolidae, Saturniidae, Thyatiridae and Zanolidae) were represented by from one to seven species.

Approximately the same number of species per iily were obtained at site 2 although only 307 Noctuidand 140 Geometridae were collected.

Large differences in family abundances as mead by the arithmetic mean number of individuals per ties were observed (Table 1). The Noctuidae were represed at both sites by the lowest average number of individuals species, while the Geometridae were next rarest. Species onging to the three intermediate-sized families were represented average by twice as many individuals as the noctuid spec while the species belonging to the smaller families wertill more abundant. Abundance values based on arithmeticans have the disadvantage that a single extremely commopecies can influence strongly the result obtained. To rule out importance of this effect in our results, the abundance of eacpecies was

**Table 2** Family differences in species abunce, based on mean log, abundance

	Si	te I	Si2			
	No. of species	Mean abundance	No. of species	Mean ibundance		
Noctuidae	401	4.12	307	3.00		
Geometridae	168	4.56	140	3.51		
Notodontidae	43	5.86	43	4.68		
Arctiidae	34	5.65	28	4.46		
Sphingidae	22	5.35	21	4.21		
Other families	32	6.16	32	4.53		

converted to a log<sub>2</sub> scale and placed in a discreabundance class or octave<sup>9</sup>; family abundance was based 1 the mean log<sub>2</sub> abundance of the species in the family. Half the species represented by just a single specimen were thus nitted from the calculations, as they belong to an incompe frequency class<sup>9,10</sup>. This omission leads to anomalous high mean abundance values in families with many species presented by single individuals, an effect which reduced the abundance differences between families in the present dat In spite of this, the same trend in family abundance was obseed (Table 2). The Geometridae were consistently more abunint than the Noctuidae, the Notodontidae, Sphingidae and actiidae were roughly 1.5 times as abundant as the Noctuie, while the other families averaged the highest abundance call.

Family differences in light trap abundance cod result from differences in phototropicity. It is difficult to sugst, however, why an orderly relationship should exist betwee the number of species in a family and the phototropicity f the species included in it. On the other hand, a relationship tween species number and 'real world' abundance is expected competitive interactions are important. Additional evidence ( competition among lepidopterans has been obtained by nalysing the effects of environmental disturbance on spec's abundance and by studying the relationship between the nurber of species in a habitat and species abundance (P.D.N.H. P.S.W., and R.H., unpublished). It seems justified to concude that the family differences in light trap abundance oberved in the present study reflect real world differences in aundance and that these differences are due to competitive interctions among species. The mechanisms by which competitio is mediated are not clear, although shared mortality facors must be important. Certainly, competition of this sort ould be intensified at the family level. A bird searching for the stick-like larva of one geometrid species might well be nore likely to discover the larva of another geometrid spcies than the bizarrely-shaped leaf-mimicking larva of a notodontid; Similarly it is known that many lepidopteran parasites are not species-specific but attack an array of related pecies11.

Our results conflict with the view that insect inpulations 12-14 are regulated largely by density-independent actors, a view which has been based on the results of case history studies of single, necessarily abundant, species. As our study suggests that the common species in a community are often phenotypically isolated from competitors, it is not surprising that

their studys resulted in a misleading impression of the importance species interactions.

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#### In vitro hagocytosis of bacteria y insect blood cells

PHAGOCYTOS is an important component of vertebrate cellular immnity and involves several stages which have been described indetail previously1.2. In contrast, little is known about this pocess in insects even though recent work3,4, on intracellular killing of bacteria indicates that phagocytosis probably plas an important role in the defence reactions of insects. In vrtebrates, the development of in vitro techniques has greatly acilitated studies on phagocytosis, but in insects such techniques are not generally available for studying the blood cells (aemocytes) in strictly controlled conditions. Here we describe or the first time the stages in the phagocytosis of

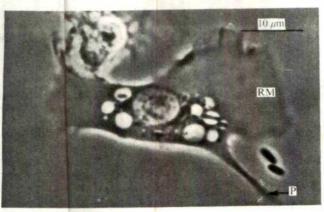


Fig. 1 Plasmatocyte with intracellular E. coli surrounded by large phagocytic vacuoles. Cell shows characteristic cell spreading, ruffled membranes (RM) and protoplasmic extension (P). Haemocyte:bacteria ratio 1:70; incubation time 30 min; phase-contrast optics.

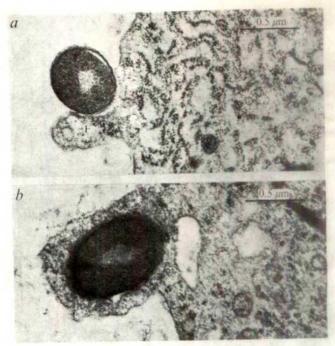


Fig. 2 Stages in attachment and ingestion of E. coli. a, Attachment of E. coli to plasmatocyte cell membrane. Note small filopodial extension (F). b, Plasmatocyte with ingested bacterium projecting from cell surface. Cells fixed in 2:1 (v/v), ice-cold 1% osmium tetroxide and 2.5% glutaraldehyde, postfixed in 0.25% uranyl acetate<sup>6</sup>. Haemocyte:bacteria ratio 1:80; incubation time 45 min.

bacteria by insect haemocytes, using a newly developed culture technique.

Final instar larvae of Galleria mellonella (120-180 mg), were bled by severing a proleg and collecting the haemolymph into Grace's insect medium (Grand Island Biological Company) to give a final haemocyte concentration of 2-3×105 ml-1. All procedures were carried out in a nitrogen atmosphere to delay the toxic effects of haemolymph melanisation. Escherichia coli K 12 (NCTC 10538), were used as test particles in bacteria: haemocyte ratios of 20-100: 1. Droplets (0.2 ml), containing haemocytes and bacteria were suspended in silicone oil in multi-dish trays (6 × 3.5 cm diameter wells; Flow Laboratories) and incubated at 26° C on an orbital shaker (45 r.p.m.) for 10-90 min. For light microscopy, the haemocytes were allowed to attach to glass coverslips after incubation, and the resulting monolayer examined unfixed.

Using previously defined criteria5, five cell types were identified in the haemolymph of G. mellonella; prohaemocytes, plasmatocytes, granular cells, spherule cells and oenocytoids but only the plasmatocytes and granular cells phagocytosed the bacteria. The plasmatocytes were easily identified by their extensive spreading on the coverslips to reveal intracellular details (Fig. 1), and had a much greater phagocytic ability than the granular cells, many of which underwent progressive degeneration in vitro.

The electron micrographs show that the characteristic attachment and ingestion phases of the phagocytic process in vertebrates2, also occurred in G. mellonella haemocytes. Initially many of the bacteria became closely attached to the haemocyte surface (Figs 2a and 3a), and the cell reacted by forming fine pseudopods (filopodia), which extended over the bacterial surface (Fig. 2a). The tips of the filopodia eventually fused with each other, or with the haemocyte surface to enclose the bacteria completely in pockets of cytoplasm (Fig. 2b). In this type of ingestion no well defined phagocytic vacuoles were formed and the bacteria frequently appeared, in low power electron micrographs, to lie directly in the cytoplasm (Fig. 3a). This 'close contact' process explains previous light microscopy reports of engulfed particles lying in the haemocyte cytoplasm

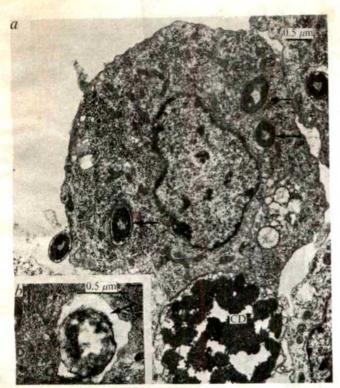


Fig. 3 Intracellular bacteria. a, Plasmatocyte with three ingested E. coli (arrows). Note lack of phagocytic vacuoles, and uptake of cell debris (CD) by invagination of cell membrane. b, Bacterium within well defined phagocytic vacuole (arrow). Fixation and incubation as in Fig. 2.

without vacuole formation7. Sometimes however, ingestion occurred by the formation of phagocytic vacuoles (Fig. 3b) similar to those described previously in infected haemocytes8. In the light microscope, these vacuoles often appeared enlarged, probably as a result of the high degree of cell spreading in the monolayers (Fig. 1).

These results confirm previous descriptions of particulate ingestion by insect phagocytes8-10 and show that bacterial phagocytosis can occur by pseudopod formation alone. The uptake of larger particles, however, such as cell debris is probably accomplished by invagination of the cell membrane (Fig. 3a) to form intracellular vacuoles.

Finally, although lysosomes are known to occur in insect haemocytes11,12, such bodies were not apparent in G. mellonella plasmatocytes. If phagocytosed bacteria are killed and degraded in the haemocytes, then the origin of the enzymes involved is unknown. We are at present utilising this in vitro technique to investigate this problem, together with other aspects of insect cellular defence reactions.

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#### Giemsa banding of metaphase chromosomes in triatomine bugs

Considerable progress has been made in developithe formal genetics and cytogenetics of several insect vectors disease1,2, notably with mosquitoes, houseflies and tsetse flies which the polytene chromosomes provide suitable materiabr detailed analysis of chromosome morphology. The trianine bugs (Hemiptera, Reduviidae) are medically important vectors of Chagas' disease in the Americas, yet cytogenetic immation on these insects is meagre<sup>3-8</sup>. These bugs present the se problems which, until recently, limited developments imammalian cytology in that they possess a large number (typidy 2n = 22) of small, almost indistinguishable chromosom. Further, since their chromosomes are also holokinetic9 (thas with nonlocalised centromeres) they do not show any imary constrictions and it is correspondingly difficult to regnise arms of a chromosome which are readily seen in chrosomes of organisms possessing discrete centromeres. This ficulty with triatomine material has now been overcome bypplying the Giemsa staining methods, which have been effective developed by mammalian cytologists10-13, to embryonic cellsf bugs with metaphase configurations. The technique I describbere makes possible the identification of individual chromomes within the complements of different species of triatominoug.

The procedure is as follows: 5-7-d-old embryosre dissected out from bug eggs, placed in hypotonic sodium ciate solution (1% for 8 min) and the cells dispersed in 0.25, trypsin in

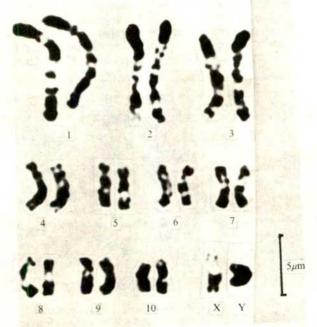


Fig. 1 Metaphase neuroblast from a Triatoma infestans male embryo 6 d old (20 + XY).

0.02% ver. The suspension is washed in insect ringer, centrifuged fixed in alcohol-acetic acid. Slides are prepared by air dryin the normal way14. Dry slides are kept for 1 week at 20° C bre heating at 60° C for 1 h in 2 × SSC (0.3 M sodium chde; 0.03 M trisodium citrate). After rinsing in absolute et ol, preparations are stained in 2% Giemsa (Gurrs 'improved'56) at pH 6.8 for 30 min, dried and mounted. This ASG (acetaline-Giemsa) technique10 has proved to be a more relialmethod for the production of G bands in bug chromoson than other methods which have also proved effective winammalian cells.

Figure hows banding and differentiation of metaphase chromoson in Triatoma infestans, and indicates the results obtainable h this technique. Similar preparations have been obtained fother Triatoma species and also Rhodnius prolixus. Preliminaryalyses indicate that there are prospects for production of romosome maps of triatomine species which could be dvalue in further investigations of chromosome markers artheir application to problems in genetic characterisation a manipulation in the vectors of Chagas' disease.

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## Forced association between higher plant and bacterial cells in vitro

Symbiotic associations with microorganisms enable many plant species to utilise molecular nitrogen<sup>1-3</sup>. In vitro systems have been developed to study some of these natural associations<sup>4-7</sup>, and here we have attempted to define an experimental system for investigiting the possibility of extending the nitrogenfixing symbosis to additional crop species. We used tissue culture techniques to force an association between the freeliving nitrogen-fixing bacterium, Azotobacter vinelandii and cells of a carrot, Daucus carota cv. Danver's Half Long. The resulting composite callus proliferates slowly on a defined synthetic medium lacking combined nitrogen. In these conditions carrot cells which have not formed an association with Azotobacter are unable to survive.

To form the Azotobacter plant cell association, carrot cell suspensions growing logarithmically in a liquid medium<sup>8</sup>

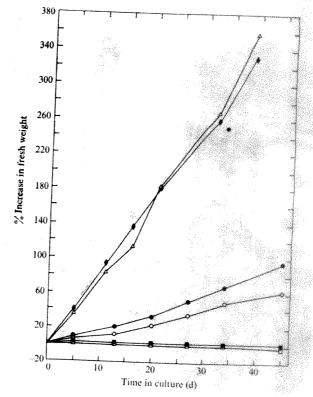


Fig. 1 Growth of Azotobacter-infected and uninfected control carrot tissues on media containing different levels of combined nitrogen. Carrot cells were grown on a Linsmaier and Skoog medium, supplemented with 4% sucrose and containing 3 mg l<sup>-1</sup> indoleacetic acid and 3 mg l<sup>-5</sup> (γ, γ-dimethylallylamino)-purine (2iP). Media were solidified with 1% agar. NH<sub>4</sub>NO<sub>3</sub> and KNO<sub>3</sub> were omitted from N-free medium. Medium which lacked NH<sub>4</sub>NO<sub>3</sub> and contained 0.19 g l<sup>-1</sup> KNO<sub>3</sub> is referred to as low N medium. The adenine autotrophic stepin of feathers. medium. The adenine-auxotrophic strain of Azotobacter vine-landii (ATCC 25308) was grown on modified Burke's nitrogenfree medium (pH 7.8) comprising Na<sub>2</sub>HPO<sub>4</sub> (0.189 g), KH<sub>2</sub>PO<sub>4</sub> (0.011 g), MgSO<sub>4</sub>.7H<sub>2</sub>O (0.20 g), FeSO<sub>4</sub>.7H<sub>2</sub>O (6.0 mg), NaMoO<sub>4</sub> (0.01 g), MgSO<sub>4</sub>, H<sub>2</sub>O (0.20 g), SrCl<sub>2</sub>, 6H<sub>2</sub>O (10 mg), NaCl (10 g), HaHCO<sub>3</sub> (50 mg), adenine HCl (20 mg) and NaCl (10 g), HaHCO<sub>3</sub> (50 mg), adenine HCl (20 mg) and sucrose (20 g) per l H<sub>2</sub>O. All calluses were grown on low N medium for 3 weeks before being transferred to the medium indicated. Initial inoculum weight was approximately 50 mg. Control carrot tissue transferred to N-free ([]), low N (18) or standard Linsmaier and Skoog ((A)) medium. Azotobacter-containing carrot tissue transferred to N-free (()), low N (1), or standard Linsmaier and Skoog (\*) medium.

containing standard nitrogen levels were inoculated with cells of an adenine-requiring strain of A, vinelandii to a final concentration of 106 ml<sup>-1</sup> bacterial cells. After 12 d the mixed cultures were washed and suspended in N-free carrot medium. The cultures were incubated for an additional 2 weeks after which they were plated in N-free carrot medium solidified with 1% Noble agar. The plates were incubated at 23° Coin a 16 h light/8 h dark cycle. The rare and slowly growing colonies were selected as they appeared 3-6 months after plating and were transferred to either N-free or low N medium. The rate of colony formation on N-free medium was approximately 10-4 to 10-5 of the total number of carrot cells plated. Control experiments in which carrot cultures were not inoculated with Azotobacter produced no colonies on N-free medium. Several independent lines of evidence demonstrate that the infected plant cultures depend upon Azotobacter for growth in the absence of combined nitrogen.

• Carrot cells are unable to survive in vitro in the absence of combined nitrogen (Fig. 1). When 1.9 mM KNO<sub>3</sub> is supplied as the sole source of combined nitrogen carrot cells do not proliferate over a 4 month period. On these media calluses which have been recovered after inoculation with Azotobacter cells increase in fresh weight. On medium containing normal

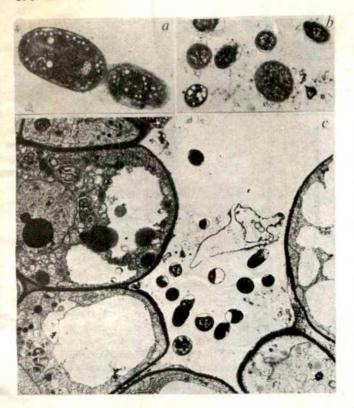


Fig. 2 Electron micrographs showing, a, the fine structure of the bacteria in the composite callus (×13,036); b, bacterial cells in the agar underlying the composite callus (×2,743); c, composite callus growing on low N medium (×2,305). Fixation of callus cultures growing on low N medium for electron microscopy was accomplished by flooding the agar plates with a 3% buffered solution of glutaraldehyde for 4 h, followed by a 6% solution of glutaraldehyde. After 4 d samples were excised from the calius and the underlying agar, washed, and postfixed in a 2% solution of OsO<sub>4</sub> overnight. The fixing solutions and wash were buffered with 0.05 M sodium cacodylate at pH 6.8. The samples were dehydrated in a graded series of acetone solutions at 0–2° C., and embedded in an epoxy resin. Thin sections were stained with uranyl and lead salts before viewing in the electron microscope.

levels of NH<sub>4</sub>NO<sub>3</sub> and KNO<sub>3</sub> (20.6 mM and 18.8 mM, respectively) no difference in growth rates is observed between uninoculated calluses and inoculated calluses which are capable of growth on N-free medium. Inoculated calluses have been cultured for over 18 months and have maintained the ability to grow in the absence of combined nitrogen during that period.

The addition of penicillin G to the medium at a concentration which is known to kill *Azotobacter* cells (50.0 µg ml<sup>-1</sup>) destroys the ability of the inoculated callus to grow on N-free medium. Growth of either inoculated or uninoculated calluses on medium containing normal levels of combined nitrogen is not inhibited by the addition of penicillin. These observations indicate that the ability of the inoculated callus to grow on N-free medium is dependent on the presence of functional bacterial cells in the callus mass.

Composite calluses capable of growth on N-free or low N medium and control calluses were suspended in T broth and in Burke's minimal and adenine-supplemented media. After several days turbidity developed only in the adenine-supplemented Burke's medium. This suspension was plated and identified as the original Azotobacter adenine auxotroph with which the calluses had been inoculated. None of these three media became turbid when inoculated with control carrot callus. The absence of growth in T Broth (which supports growth of a prototrophic Azotobacter strain, but not of the adenine-requiring strain) after a 3 week period indicates that the calluses contain no contaminating microorganisms.

Electron micrographs of inoculated carrot callus grown on low N medium clearly establish the presence of bacterial

Table 1 Acetylene-reduction activity

	Incubation period (h)	$nmolC_2H_4$ pegas $\pm$ s.d.
No tissue	10	0.22-5
Azotobacter	1	1.46 6
Carrot callus	10	0.26 0
Carrot-Azotobacter callus	0	0.25 1
Carrot-Azotobacter callus	10	0.71 )7

Approximately 50 mg fresh tissue was placed in 1 nals which were then injected with 0.10 ml acetylene. The Azotobr controls contained approximately  $8 \times 10^7$  cells in 0.10 ml mod Burke's medium. Reactions were stopped by the addition of ml 0.1 N H<sub>2</sub>SO<sub>4</sub>. The ethylene content of samples was determine published gas chromatographic procedures<sup>11</sup>. The composite ca does not evolve ethylene in the absence of acetylene. It is presid that the level of ethylene found in the absence of tissue wassent as a contaminant in the acetylene used in the a/.

cells in the intercellular regions of the tissue (Fig. 2nd in the underlying agar (Fig. 2b). Live bacteria were robserved within the carrot cells. The fine structure of the baca growing with the callus cultures (Fig. 2a) was comparable in essential details to Azotobacter vinelandii<sup>9,10</sup>. No other orgisms were found with the callus or in the agar.

Azotobacter-containing carrot tissue capable growth on N-free medium evolves significantly more ethne in the acetylene assay for nitrogenase than does an inoculated control callus (Table 1).

The association between cells of carrot and Azlbacter has been accomplished by establishing conditions mutual dependency in culture. Presumably, carrot cells reve reduced nitrogen from Azotobacter cells which, in turn, pend upon the carrot cells to satisfy their auxotrophic requement for adenine. This association is not completely stable casionally the callus segregates some cell masses which he lost the ability to grow on N-free medium while others retathis ability. Other calluses become overgrown by bacterialells. Most of the composite calluses (>90%), however, havmaintained a stable association over the 18 months they we been in culture.

An assessment of the usefulness of the carroidzotobacter association and of the magnitude of its effectmust await regeneration of plants from the composite callus and determination if the association is maintained in who plants. Up to now we have been unable to accomplish plantegeneration from these calluses.

The use of genetic markers and nutritional regirements to establish complementary associations between ifferent cell types should be a versatile procedure applicable to diverse experimental situations. The cellular associatin reported in this work might serve as a first step in the deslopment of new or more general symbiotic relationships.

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#### Chromatin attachment to nuclear membrane of wheat pollen mother cells

THE cellular mechanisms through which homologues recognise each other before meiosis are unresolved. The problem is compounded in a polyploid species like Triticum aestivum which contains three diploid sets of genetically similar chromosomes1. At meiosis, pairing normally takes place only between the fully homologous partners of a set, so that in spite of their close genetic similarity, homeologues do not synapse. The absence of homœologous pairing depends principally upon the presence of chromosome 5B (ref. 2) and probably derives from the operation of the Ph locus which is distally located on the long arm of that chromosome3.4.

Recently there has been increased interest in premeiotic development in wheat<sup>5</sup> and it has become clear that activities occurring during premeiotic interphase are critical to chromosome alignment<sup>6,7</sup>. As such alignment might be effected by a structural mechanism we undertook a preliminary investigation of the ultrastructure of the wheat pollen mother cells (PMCs) at premeiotic interphase. Here we report our observation of fibrous material which seems to link chromatin to the nuclear membrane during premeiotic interphase, and discuss the possible significance of this material.

Plants of T. aestivum (var. Chinese Spring) were transferred to a constant environment at 20° C with continuous light, about 2 weeks before meiosis in leading tillers. When tillers reached therequired developmental stage they were excised and transferred to the laboratory. The three anthers were quickly excised from each of several florets. One anther from each floret was fixed in 1:3 acetic acid and a Feulgen stained squash preparation prepared. Appropriate florets were identified from cytological examination of these squashes using the criteria previously described5. The remaining two anthers from such florets were fixed in 5% glutaraldehyde and prepared for ultrastructural studies. As the three anthers within a floret are approximately synchronous in development8, those used for ultrastructural studies should contain PMCs at the same stage as the Feulgen-stained samples. Nevertheless, to rule out possible mistakes due to variation between anthers, the stage of development was rechecked using thick and thin sections stained with methyene blue and osmium, respectively. The checks in squashed and sectioned preparations were based on the same criteria. A description of the sequence and appearance of the stages examined has been given previously5.

A distinct and novel feature of the ultrastructure of premeiotic cells was the presence of bundles of fibrillar material in the PMC nuclei Individual fibres had a diameter of about 200 Å. Such fibrillar material was observed adjacent to the nuclear membrane apparently forming attachments at intervals between the membrane and chromatin masses (Fig. 1a and b). Bundles of fibrillar material were also found well within the nucleus. Figure 1d shows one such bundle about 1.2 µm long

and 0.2 µm wide apparently attached to chromatin at one end. Similar fibrillar material was not found in any other anther cells even though it was a general and constant characteristic of the PMCs. The exclusive presence of these fibres in the premeiotic but not in the adjacent somatic cells makes it likely that they are exercising some specific meiotic function.

The order of appearance of these fibrils relative to that of other meiotic nuclear structures is particularly relevant to speculation about their function. The relationship is illustrated diagrammatically in Fig. 2. The fibrils appear in the nuclei of premeiotic cells well before the appearance of any lateral elements and also before the investiture of the cells with callose, another distinctive characteristic of plant meiocytes. The presence of the fibrils overlaps the appearance of lateral elements and of the synaptinemal complex. The fibrils do not persist over the life time of the synaptinemal complexes. They were absent from sections of PMCs that had developed beyond 'stage 3'. At, or soon after 'stage 3', paracrystalline bodies were seen adjacent to the nuclear membrane (Fig. 1e). These may represent a step in the reorganisation or in the degradation of fibrillar material.

Some details of the changes occurring within the neighbourhood of the nuclear membrane are of interest in connection with the relationship of the nuclear membrane to chromosome synapsis, a relationship for which circumstantial evidence is available in a variety of studies8-10. Before and during 'stage 1' a large proportion of the nuclear membrane was in contact with

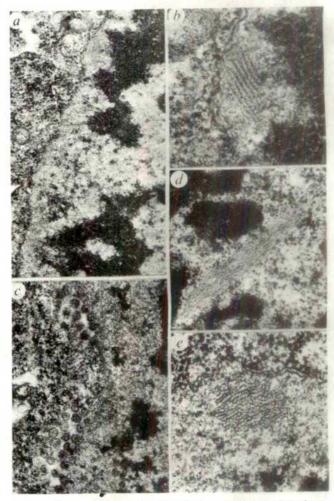


Fig. 1 Electron micrographs of PMCs from Chinese Spring wheat showing: a, fibrillar material located between the nuclear membrane and chromatin masses (× 28,700) at 'stage 215; membrane and chromatin masses (× 28,700) at 'stage 2's, b, a higher magnification (× 71,820) of fibrillar material from a; c, pores in the nuclear membrane at 'stage 2' (× 28,700); d, a large mass of fibrillar material located within the nucleus but away from the nuclear membrane ( $\times$  33,600) and,  $\varepsilon$ , a paracrystalline body adjacent to the nuclear membrane at 'stage 3' (× 57,400).

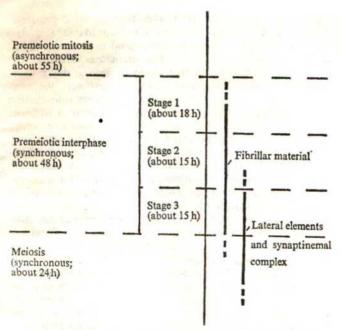


Fig. 2 The time and sequence of appearance of fibrillar material, lateral elements, and SC during premeiotic interphase and meiosis in PMCs of Chinese Spring wheat grown at 20° C.

chromatin either directly or through the fibrous material. Throughout this period the nuclear membrane had very few obvious pores. Early in 'stage 2' it became punctured by numerous pores (Fig. 1c) without, however, removing the fibrous material. Also, during 'stage 2' a zone about 2,000 Å wide containing little or no chromatin appeared next to the nuclear membrane (Fig. 1a and c). This zone, which differed in appearance from the rest of the nucleoplasm, extended around the whole circumference of the nucleus and contained in addition to the fibrillar material numerous structures, less defined but nevertheless sufficiently similar to suggest their functioning as agents of interaction between the nuclear membrane and chromatin.

The discovery of synaptinemal complexes revealed the nature of the mechanism that apparently stabilises meiotic chromosome pairing. The means by which homologues are suitably juxtapositioned before synaptinemal complex formation remain unknown, however. Some organisational framework would seem to be necessary for facilitating the prealignment of chromosomes for synapsis. We have found bundles of fibrillar material apparently forming long range connections with chromatin (Fig. 1d) and also linking chromatin to the nuclear membrane (Fig. 1c). It is noteworthy that the appearance of fibrillar material coincides with the interval when chromosome synapsis in wheat is sensitive to the action of colchicines or to low temperature7. Equally noteworthy is the fact that this interval begins before the initiation of synapsis and of lateral element, or synaptinemal complex formation. Colchicine has been shown to interfere with the normal functioning of the nuclear membrane during meiotic prophase in Lilium8 and, therefore, it may be significant that an appreciable fraction of the fibrils in wheat has been found associated with the nuclear membrane. We suggest, therefore, that the fibrillar material found only in PMCs at stages 1 to 3 may function in establishing or maintaining the spatial coorientation of chromosomes which is a prerequisite for normal meiotic pairing in wheat, and also that it may contain colchicine sensitive protein.

The appearance of fibrillar material before the first synaptinemal complex formation, and its disappearance soon after, would fit this hypothesis. If fibrillar material is responsible for long range links it would presumably cease to be useful once stable links are established between homologues unless the fibrils themselves become incorporated in the synaptinemal complex.

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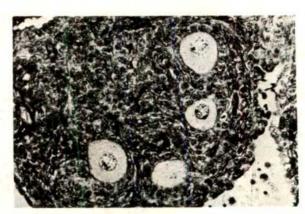
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#### Does the rete ovarii act as a trigger for the onset of meiosis?

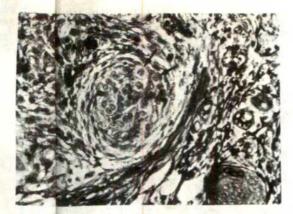
ALTHOUGH transformation of oogonia to ooytes in the mammalian ovary and the subsequent formation of follicles have been much studied the factors which initiate meiosis remain obscure and the control of the formation of the granulosa layer is poorly understood.

Experimental results presented here indicate hat the rete tubuli is important for the onset of meiosis and hat the early formation of the granulosa layer is dependent on the attachment of the rete system to the ovary. Moreover, normal growth or maintenance of the ovarian tissue itself seem to le dependent on the extraovarian rete tubules.

The morphological similarity between the rete carii and the rete testis was already recognised in the last century1. Both consist of tubules lined with cuboidal or cylindrial epithelial cells, which arise from the mesonephros. Duringearly follicle formation a continuity between the tubes from tle rete ovarii



Intact foetal mouse ovary (12 d post :oitum) after inoculation in nude mouse for 14 d. Four apparently normal growing oocytes are seen (× 423) (Stain: Azan).



Cal part of a 12-d-old mouse ovary without the rete tubules af 14 d inoculation into nude mouse. The graft contains o oogonia (arrows) and no follicle cells. (× 423) (Stain: Azan).

and the folles has been seen2-6. Kölliker2 and Byskov and Lintern-Moe proposed that cells of the rete ovarii contributed to ficle formation.

To test perimentally whether the onset of meiosis is dependent othe rete ovarii, we used ovaries from two groups of mouse emyos. As the transformation of oogonia to oocytes occurs in thmouse between the 13th and the 17th day of embryonic lil.8, one group was aged 12 d post coitum, in which the rete tube had not yet invaded the gonads, but were lying entirely outse the ovary as extraovarian rete and the meiotic prophase hanot started; in the second group, aged 15 d post coitum, the te was present within as well as outside the ovaries and le major part of the germ cells had entered the meiotic propase. Under a dissecting microscope three ovaries in each grou of six were divided into a cranial part with the adherent extlovarian rete tubules and mesonephric remnants and a caudalart of 'pure' gonadal tissue, without extraovarian rete tubules. The three other ovaries in each group were left intact. In oler to study the transformation of oogonia to oocytes in goads with and without the presence of the extraovarian retetubules the intact ovaries and the cranial and

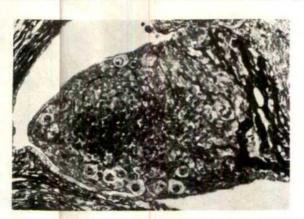


Fig. 3 Caulal part of a 15-d-old mouse ovary without the rete tubules afte 11 d implantation in nude mouse. Some oocytes are seen in the periphery of the graft. No follicles have formed. Necrotic changes are seen in the centre of the graft. (× 423) (Stain: Azan).

caudal parts of ovaries were inoculated subcutaneously into adult female mutant nude mice with aplasia of the thymus (strain BALE/c Nu/Nu). This method of grafting has already

The embryonic ovarian tissue was removed from the nude mice and prepared for histological study after 14 and 11 d respectively. At removal the grafted gonads corresponded to a 5-d-old mouse ovary.

Intact grafted ovaries and the cranial part of the ovary with

the rete tubules attached, both taken from 12-d-old foetuses, contained small and medium sized follicles at 'day 5' (Fig. 1). Their oocytes were indistinguishable from oocytes seen in a normal 5-d-old mouse ovary. The granulosa layer of the follicles was more irregular than normally seen at day 5 with many finger-like extensions to other follicles or to the rete system. Necrotic changes did not occur in these transplants.

The caudal part of the 12-d-old embryonic ovaries without the extraovarian rete tubules grafted for 14 d contained only oogonia (Fig. 2). Oocytes and follicles were never seen. The oogonia were surrounded by undifferentiated fibroblast-like cells. In two of the three grafts necrotic changes were observed. In contrast to this the cranial part of the ovary with the extraovarian rete tubules attached contained oocytes as well as follicles after inoculation for 14 d.

All the ovaries grafted from the 15-d-old embryos, both with and without the extraovarian rete tubuli attached, contained oocytes after implantation for 11 d. Intact ovaries as well as the cranial part of ovarics developed follicles, which corresponded in size to those found in a 5-d-old mouse. None of those grafts showed necrotic changes. The caudal part of the ovary without the rete tubules consisted only of a mass of undifferentiated cells with oocytes lying closely together in group at the periphery (Fig. 3). Also in these rete-depleted grafts necrotic changes were seen in two cases. No follicular formation had occurred.

Recent ultrastructural studies show that the extraovarian rete cells are very active holocrine secreters. Whether the effect of the rete system depends on direct cellular contact with the germ cells or on a trigger component secreted from the rete cells remains still uncertain.

Preliminary studies of implantation of ovaries from rabbits and hamsters support our results. The rete ovarii is essential for the start and maintenance of meiosis and for further ovarian development.

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#### How selective is high affinity uptake of GABA into inhibitory nerve terminals?

It is now well established that brain slices and homogenates possess distinct high affinity uptake systems for all the major transmitter candidates or their immediate precursor molecules1,2, In the case of GABA and glycine the application of electron microscopic autoradiography to such in vitro preparations has revealed discrete populations of nerve terminals as the major site of amino acid uptake3. In brain slices or homogenates, however, nothing is known about the functional or

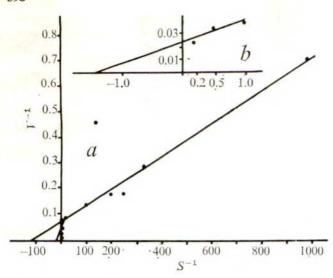


Fig. 1 Lineweaver Burk plots from a single experiment to show high and low affinity uptake of  ${}^{3}\text{H-GABA}$  into cerebellar glomerulus particles. The kinetics of the uptake were characterised by two straight lines, the shallower of which described a high affinity system with an apparent  $K_{\rm m}$  and  $V_{\rm max}$  of 9.6  $\mu$ m and 15.6 nmol per mg protein per 10 min respectively and the steeper (a, shown in more detail above) a low affinity system with a  $K_{\rm m}$  and  $V_{\rm max}$  of 780  $\mu$ M and 46.2 nmol per mg protein per 10 min (S= GABA concentration in nM and V= velocity in nmol per mg protein per 10 min). The glomerulus particles (320  $\mu$ g protein) were incubated at 37° C for 10 min in 1 ml of an isotonic salt medium, buffered with Tris-HCl, pH 7.4 and containing 32 mM of glucose and 10  $\mu$ M amino-oxyacetic acid. The size of the inulin space and  ${}^{3}\text{H-GABA}$  concentration of the particles was estimated by the method of Levi and Raiteri ${}^{13}$ . The experimental points are the means of duplicate estimates and the lines were plotted by computer analysis.

anatomical identity either of the labelled or the unlabelled populations of terminals. In other words, it has not hitherto proved possible to confirm that each high affinity uptake process for any particular transmitter substance is present exclusively in those nerve terminals in the brain known from independent neurophysiological studies<sup>4</sup> to release that substance.

Fortunately, the newly perfected technique for isolating large pieces of the cerebellar glomeruli with well preserved ultrastructure (glomerulus particles)5-7, now offers a preparation suitable for testing the two fundamental propositions of this hypothesis (at least for the putative transmitter GABA) namely that the high affinity uptake system is exclusively present in recognisable inhibitory endings which are known to utilise GABA as their transmitter substance and that the uptake is completely absent from other categories of nerve terminals. The inhibitory nerve terminals of the cerebellar glomeruli arise from Golgi cell axons8, and form one of the two inputs on to the granule cell dendrites. These inhibitory terminals are adjacent to the excitatory endings of the mossy fibres, which can easily be distinguished from the Golgi cell axon terminals by their large size and other ultrastructural features8.10 (Fig. 2).

The presence of a high affinity uptake process for  ${}^{3}\text{H-GABA}$  in the glomerulus particles was demonstrated in three separate experiments in which glomeruli were incubated in the presence of a wide range of GABA concentrations (1–5,000  $\mu$ M) (see Fig. 1). The  $K_{m}$  values obtained (between 7 and 12  $\mu$ M) were almost identical to those reported in slices of cerebral cortex. In addition,  ${}^{3}\text{H-GABA}$  was also taken up by a second low affinity uptake process which was characterised by a  $K_{m}$  of 780  $\mu$ M. The tissue components responsible for the high affinity uptake process were identified by electron microscopic autoradiography carried out on glomerulus particles incubated in the presence of a low concentration of  ${}^{3}\text{H-GABA}$  (2.5  $\mu$ M), thus avoiding any appreciable uptake by the low affinity process (Fig. 2). In spite of incubation in media containing

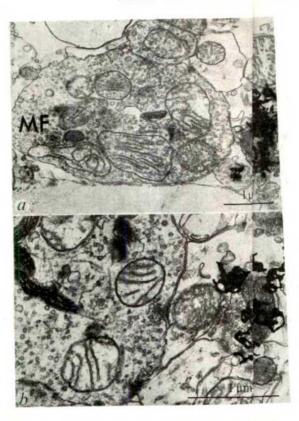


Fig. 2 a, Electron microscopic autoradiograph a lomgerulus particle showing the marked contrast lween the mossy fibre terminal (MF) which is completely ded of silver grains and the small Golgi axon on the right one picture which is overlaid by an intense cluster of silver grainb, A small portion of another glomerulus particle in which thunlabelled mossy fibre terminal and granule cell dendrite is shon to be in close morphological proximity to the intensely lalled Golgi axon terminal. The glomerulus particles (about 1-ng protein derived from six rat cerebella) were incubated in thoresence of 2.5 µM <sup>3</sup>H-GABA (specific activity 10 Ci mmol at 37 C for 6 min in 1 ml of buffered saline. After inevation, the glomeruli were centrifuged, washed, fixed in 5% glaraldehyde and processed for autoradiography<sup>3</sup>.

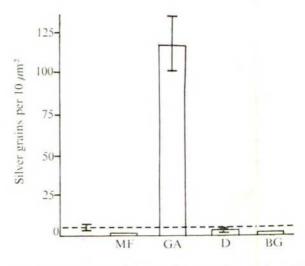


Fig. 3 Distribution of silver grains over electronmicroscopic autoradiographs of isolated cerebellar glomeruli abelled with  $^3\text{H-GABA}$ . The histogram (mean  $\pm$  s.e.) from a analysis of 15 different electron micrographs, each containing one complete glomerulus particle, to show the silver grain densityas a function of area for the various identifiable components, the mossy fibre terminals (MF), the Golgi axons (GA), the granulecell dendrites (D) and the background (BG). The dotted line shovs the average density of silver grains per micrograph, each d which was  $8\times10$  inches and represented an area of 60 q  $\mu m$ .

saline, the structure of the glomerulus particles was well

Intense cirs of silver grains resulting from the accumulation of 3H BA were found almost exclusively over small nerve termi which closely resemble the Golgi axon terminals charrised by Palay and Chan-Palay<sup>10</sup> in sections from in sitted adult rat cerebellum. Such grain clusters were never a over the adjacent mossy fibre terminals. To emphasise (selectivity of the uptake into inhibitory, as opposed to itatory terminals, a statistical analysis of the silver grain ribution was carried out on 15 electron micrographs, eacof which contained a complete glomerulus particle (Fig. The silver grain density over the Golgi axons was more tl 20 times higher than the average density per micrograph more than 100 times greater than the grain density ovehe mossy fibre terminals. The postsynaptic granule cellendrites, like the excitatory terminals, were virtually dev of silver grains.

As well aonfirming the hypothesis set out in the introduction these sults taken in conjunction with the previously reported contration of glutamic acid decarboxylase in the glomerulus picles<sup>6,7</sup> and its presence in Golgi axon terminals in situ11 suge that these inhibitory terminals retained their in vivo propes. This view is strongly supported by a parallel study12 in vch a microinjection of the GABA analogue <sup>3</sup>H-2, 4-diambutyric acid was shown to cause an identical

distribution silver grains to occur in vivo.

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### Glutamatereceptors in the rat central nervous system

ALTHOUGH it is widely accepted that glutamate may be a significant central transmitter (for reviews, see refs 1 and 2), definitive neuropharmacological evidence at the synaptic level has not been forthcoming, and studies with proposed glutamate antagonists3-6 lave yielded anomalous results. On the basis of biochemical evidence, however, glutamate fulfils many of the criteria expected of a neurotransmitter; in particular, nerve endings8 and glia9,10 possess high-affinity transport systems for glutamate, which it is thought, in common with other amino acid neurotransmitters, are responsible for terminating its synaptic actions. For these high-affinity systems to function, there is a stringent requirement for sodium<sup>11</sup>, which may be involved in the initial binding phase at the reuptake site, prior to transport12-14. Until recently, very little attention has been paid to the possibility of investigating directly the biochemical properties of postsynaptic amino acid receptors. Young and

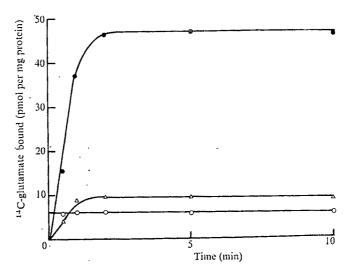


Fig. 1 Time course of binding of 14C-glutamate to synaptic membranes. Male Sprague-Dawley rats were decapitated and cerebral cortices homogenised in 20 vols ice-cold 0.32 M sucrose (Teflon homogeniser, 0.25 mm clearance, 800 r.p.m.) and centrifuged at 1,000g for 10 min. The supernatant was then and centrifuged at 1,000g for 10 min. The supernature was their centrifuged for 20 min at 17,000g to give a crude mitochondrial pellet (containing mainly myelinated axons, mitochondria and synaptosomes). The pellet (P<sub>2</sub>) was resuspended in 1.2 ml 0.32 M sucrose, which was layered on to a discontinuous gradient consisting of: 4 ml 0.8 M sucrose, 4 ml 1.2 M sucrose and 2 ml 1.5 M sucrose. Gradients were centrifuged in 6×15 ml consisting 01: 4 mi 0.8 M sucrose, 4 mi 1.2 M sucrose and 2 mi 1.5 M sucrose. Gradients were centrifuged in  $6 \times 15$  ml swing-out heads for 60 min at 112,000g. The synaptosomes were collected at the 0.8-1.2 M sucrose interface, lysed with 20 vols ice-cold distilled water in a glass homogeniser and the homogenate centrifuged for 20 min at 9,000g. The supernatant and the soft pellet were removed and recentrifuged at 50,000g. for 60 min the resulting pellet resupposed in 50,000g for 60 min, the resulting pellet resuspended in Tris-Krebs buffer, pH 7.4 (containing 50 mM Tris substituted for bicarbonate, and either 115 mM sodium chloride or 230 mM sucrose) and aliquots taken for protein estimations<sup>17</sup>. Membrane suspensions  $(6.0-6.5 \text{ mg protein ml}^{-1})$  were stored at  $-30^{\circ}$  C, no significant loss of binding activity was detected after 2 months.

Synaptic membranes (0.31-0.35 mg protein ml-1) were incubated in 0.5 ml Tris-Krebs medium (with or without sodium<sup>+</sup>) at 25°C and containing 1.75×10<sup>-6</sup> M L-U
<sup>14</sup>C-glutamate (Amersham) for periods of time from 30 s to <sup>14</sup>C-glutamate (Amersham) for periods of time from 30 s to 10 min. Membranes were recovered by rapid (approx 5 s) vacuum filtration through Whatman GF-C glass fibre filters followed by rinsing with 5 ml Tris-Krebs. Filters were transferred to vials and the tissues digested with 0.4 ml Soluene-100 (Packard) prior to addition of fluor for liquid scintillation counting. To correct for binding of <sup>14</sup>C-glutamate at sites other than for uptake and the postsynaptic receptors (that is, nonspecific binding), the binding of <sup>14</sup>C-glutamate was studied in the presence of a large molar excess of unlabelled L-glutamate. This nonspecific binding was not saturable and increased proportionately with increasing <sup>14</sup>C-glutamate increased proportionately with increasing <sup>14</sup>C-glutamate concentration. In all experiments to determine specific glutamate binding, this nonspecific component was subtracted. The effect of synaptic membrane protein concentration on specific 14C-glutamate binding was also investigated and was found to be linear over the range studied (0.2-0.5 mg protein ml<sup>-1</sup>).

•, Specific <sup>14</sup>C-glutamate binding in the presence of sodium;

△, specific <sup>14</sup>C-glutamate binding in sodium-free medium;
⊙, nonspecific <sup>14</sup>C-glutamate binding (blank values, <sup>14</sup>C-glutamate bound to filters in absence of tissue subtracted from each value) determined in the presence of unlabelled glutamate  $(5 \times 10^{-3} \text{ M})$ . Results are means of quadruplicate determinations; s.e.m. all <10%.

Snyder<sup>15</sup> have demonstrated that strychnine, a potent and selective antagonist of glycine-induced hyperpolarisations of spinal neurones, binds specifically to a component of synaptic membranes; this is probably the physiological glycine receptor since strychnine has negligible affinity for the glycine highaffinity uptake system. The specific γ-aminobutyric acid (GABA) antagonist, bicuculline, has also been found to competitively inhibit GABA binding to synaptosomes, in which the uptake site had been inactivated by chlorpromazine 16. In both these cases, binding to the postsynaptic receptor was not affected by the absence of sodium.

I have therefore examined the possibility that in the absence of sodium, the characteristics of the still hypothetical, specific glutamate receptor on synaptic membranes might be investigated.

Figure 1 shows the time course of specific 14C-glutamate binding at 25° C and, in common with the binding of strychnine to glycine receptors, nonspecific binding was not time dependent (essentially instantaneous), whilst the specific binding was rapid, with a plateau after 2 min. Half maximal binding occurred within 30 s, both in the presence and absence of sodium. When binding was studied at 0° C, maximal binding was not apparent for 8 min. All further binding studies were carried out with a 5 min incubation period.

Kinetic characteristics of specific glutamate binding were then examined under two experimental conditions: (1) in normal Tris-Krebs medium containing N-methyl-pL-aspartate (10<sup>-3</sup> M), a highly potent glutamate agonist<sup>18</sup> with no affinity for the glutamate uptake system<sup>19-21</sup>; and (2) in sodium-free Tris-Krebs medium. Such a scheme should theoretically permit assessment of binding to the uptake and receptor sites individually. Binding was measured over a restricted range of  $^{14}$ C-glutamate concentrations  $(4 \times 10^{-7} - 8.7 \times 10^{-6} \text{ M})$  and

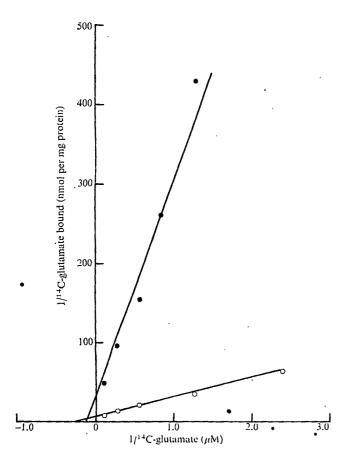


Fig. 2 Lineweaver-Burk plots of 14C-glutamate binding to cortical synaptic membranes in Tris-Krebs medium containing N-methyl-DI-aspartate (10<sup>-3</sup> M) (O) and in sodium-free medium ( ). Lines of best fit by regression analysis. Each point is the mean of quadruplicate determinations; s.e.m. all  $\leq 10\%$ .

Table 1 Pharmacological antagonism of spi 14C-glutamate binding

	Tris-Krebs		
+ Na <sup>+</sup>		- Nai	
Specific	Inhibition†	Specifi	nhibition
binding*	%	bindin'	%
$44.00 \pm 0.41$		8.83 + 0	
$40.86 \pm 0.20$	5	$7.83 \pm 0$	11‡
$3.65 \pm 0.16$	79	3.25 + 0	63§
$19.99 \pm 1.43$	428	$3.30 \pm 0$	63§
,	·	_	
38.17±0.50	0	3.52±0	60§
$34.03 \pm 0.44$	18‡	$6.46 \pm 0$	27‡
	binding* 44.00±0.41 40.86±0.20 3.65±0.16 19.99±1.43 38.17±0.50	+ Na <sup>+</sup> Specific Inhibition† binding* 44.00±0.41 40.86±0.20 5 3.65±0.16 79    19.99±1.43 428  38.17±0.50 0	Specific binding*   Mail binding*   Specific binding*   Mail binding*   Specific bin

Synaptic membranes were incubated in Tris-Krehedium for 5 min with L-U- 14C-glutamate and one of the above sances at a concentration of 10<sup>-3</sup> M.

\*Specific binding in pmol per mg protein.
†Percentage inhibition in the presence of Na+ corrector binding component to receptors (-Na+ alone).

Means ± s.e.m. of quadruplicate determinations; hificance of difference from control in each group (that is, + &-Na+) by student's t test.

 $P \leqslant 0.05$ .  $\delta P \leqslant 0.005$ .  $||P \leq 0.001.$ 

the double reciprocal plots (Fig. 2) reveal apparerissociation constants (pKs) of  $4.0 \times 10^{-6}$  M and  $8.3 \times 10$ M for the uptake and receptor sites respectively.

If there are subtle configurational differences netween the postsynaptic glutamate receptor and the presiptic highaffinity uptake site, an investigation of specific -glutamate binding to synaptic membranes in the presendbinding to both sites) and absence (binding to receptors ly) of Na+ should readily provide the means of distinguing between glutamate agonists and the highly elusive glutamaintagonists. Acidic amino acids such as N-methyl-DL-artate and D-homocysteate, with strong excitant action on central neurones, are not substrates for the high-anity uptake system19-21-a property shared only with the five corounds which have been suggested as effective antagonists | glutamateinduced and some synaptic excitations in the NS, that is, glutamic acid diethylester<sup>4,5</sup>, α-methyl-DL-glumic acid<sup>3</sup>, 1-hydroxy-3-aminopyrrolid-2-one (HA-966)6, 2-methoxyaporphine and L-methionine-DL-sulphoximine in contrast, medium excitants, such as L-glutamate, L-asparte, L-cysteate and L-homocysteate, are all potent blockers chigh-affinity glutamate uptake. Table 1 shows the effects of N+ on specific L-glutamate binding and how this can be maffed by the presence of some glutamate congeners. Binding itereospecific as evidenced by the lack of competition by D-glumate, which is a weak excitant with minimal effects on uptal, L-aspartate produced a marked inhibition of binding at bo sites, while DL-homocysteic acid (DLH) showed a greateieffect at the glutamate receptor than at the reuptake site. DLH i considerably more potent as a neuronal excitant than is eithe L-glutamate or L-aspartate18, and my results suggest that his may be attributable to the lesser ability of DLH to bindo the uptake site, than either glutamate or aspartate, since s affinity for the glutamate receptor appeared to be equivalnt to that of L-aspartate. Glutamic acid diethylester, one of the more effective glutamate antagonists, had no effect a the specific binding of 14C-glutamate at the uptake site, bt produced a highly significant inhibition of binding at thereceptor site. Finally p-chloromercuriphenylsulphonate, a sustance which at low concentrations potentiates the synaptic ations of both excitant and inhibitory amino acids, possibly by inhibiting their reuptake<sup>22</sup>, produced some reduction of speific glutamate binding at both sites. These effects were probally nonspecific, since mercurials combine extensively with the subhydryl groups of membrane components23,24.

These results support the idea that specific gluamate binding to its postsynaptic receptor may be distinguished from binding

to its uptaite by its independence of sodium. I am now investigatine effects of a wide range of potential glutamate antagonists the specific binding of glutamate, and attempts will be n to isolate and characterise these binding components has been done for the insect glutamate receptor25.

I thankt J. C. Watkins (Bristol) for his gift of N-methyl-partate.

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#### Human x mouse hybrid cells segregating mouse chromosomes and isozymes

THE usual patern of chromosome segregation in interspecific human x moue somatic cell hybrids is retention of mouse and loss of human chromosomes1. In attempts to generate hybrid strains expressing differentiated functions, we have fused an actively dividing human cell line with freshly dissociated, highly differentiated cells from the mouse embryonic nervous system. The majority of the resulting hybrid strains retain human while losing mouse chromosomes and isozymes and thus provide a new class of hybrid cell for analysis of gene linkage and regulation.

The human fibroblast parent used was WI-18-VA2 (VA2)<sup>2</sup>, a SV40-transformed clonal derivative of WI-18, deficient in

Table 1 Chromosome composition of VA2 x mouse nervous system hybrid cell lines

		Mean chromosome no. (Range)						
Cell line	Telocentric	Biarmed	Total					
Parent VA2 Normal mouse Expected	6 (1-11) 40 46	68 (52-79) 0 68	74 (66–83) 40 • 114					
Hybrid VMPTH12 VMPOC18E VMPE18F VMPOC15M VMPTH14D VMPTH18	36 (32–38) 31 (23–36) 27 (18–33) 26 (19–34) 22 (18–27) 18 (10–27)	87 (78–93) 50 (44–56) 58 (49–67) 51 (45–63) 89 (80–101) 84 (74–94)	123 (115–131) 81 (72–89) 85 (67–98) 77 (67–89) 110 (98–124) 102 (96–117)					
VME15 VME10 VMPTH28G VMSC10 VMSG5	13 (6–18) 11 (5–16) 9 (5–17) 8 (5–11) 5 (4–9)	96 (77–106) 63 (56–76) 64 (81–49) 82 (66–92) 84 (70–99)	109 (93–121) 74 (65 -84) 73 (56-93) 90 (74–107) 89 (81–103)					

Cells were grown in modified F125 medium (F) or Dulbecco's modified Eagle's medium (D), supplemented with 5% foetal calf serum and, as indicated, HAT (FHAT, DHAT), in an atmosphere of 5-10% CO<sub>2</sub>, 90-95% air with 95% relative humidity, at 37 C in Falcon plastic ware. Parental VA2 cells after growth in 100 µM 6-thioguanine-supplemented medium formed < 1 × 10<sup>-7</sup> colonics per inoculated cell in FHAT, while parental mouse pervious system. o-thioguanine-supplemented medium formed  $< 1 \times 10^{-7}$  colonics per inoculated cell in FHAT, while normal mouse nervous system cells formed  $1-5 \times 10^{-7}$  colonies per inoculated cell in FHAT. Trypsinised, washed, VA2 cells  $(3 \times 10^{9})$  were mixed with normal mouse cells  $(10^{4}-5 \times 10^{9})$  in serum-free medium (1 ml) and fused with 200–500 haemagglutinating units (0.03% chick red blood cells of  $\beta$ -propiolactone-inactivated Sendai virus, 10 µg DNase I, as before Washed, fused cells were either diluted immediately in medium into many 100 mm dishes or seeded into one dish and then trypsinised and many 100 mm dishes or seeded into one dish and then trypsinised and distributed 24 h later. All cells were transferred to FHAT medium 24 h after fusion, fed every 4 d with FHAT, and hybrid colonies were isolated 18-24 d later as independent mating events by trypsinisation in penicylinders (Fischer Scientific Co.). Many lines were recloned before testing (indicated by letter suffixes of clone name). Cells were about trypsinisation expensionally case a week and interpretable to the control of the control o subcultured by trypsinisation approximately once a week and intermittently stored in the vapour phase of a N<sub>2</sub> freezer with 7.5% dimethylsulphoxide (DMSO) or 5% DMSO and 5% glyccrolsupplemented medium Chromosomo provides and the control of the contro supplemented medium. Chromosome preparations were prepared by arresting logarithmic phase cells (50-100 doublings after fusion) in 0.02 µg ml<sup>-1</sup> colcemid for 4 h, swelling in 75 mM KCl, air drying and then scoring 20-40 metaphases per cell line using a camera lucida attachment on a Wild microscope. Embryonic mouse brain (18 d in utero) was dissociated, cultured in DHAT for 2 weeks, and then scored. Eighty-six per cent of the cells had 40 chromosomes, 2% had 37, 6% had 50, and 6% had 80 chromosomes.

hypoxanthine phosphoribosyltransferase (HPRT) and thus unable to grow in HAT-supplemented medium3. A suspension of parental mouse cells was obtained by dissecting specific anatomical regions of C57BL/6 or NIH/Swiss-GP 15-18 d in utero embryonic mouse nervous systems, pooling the same regions from one litter, and then dissociating cells in trypsin, collagenase and DNase4. Cells were used for fusion directly (VM series) or incubated overnight in complete medium and the non-attached cells collected (VMP series).

Using Sendai virus (legend of Table 1) hybrid cell lines were generated repeatedly in fusions between human VA2 cells and cells freshly derived from the embryonic mouse, rat or Chinese hamster nervous system resulting in the same pattern of reverse chromosome segregation; however, only data on human-mouse hybrids are presented here. Approximate yields were  $3 \times 10^{-5}$  hybrid colonies per VA2 parent and  $2 > 10^{-5}$  per normal rodent nervous system cell put into the fusion. The cells appeared fibroblastic, not similar in morphology to mouse neuroblastoma cells6, and all were negative when stained acetylcholinesterase<sup>6</sup> (1,850 colonies scored). The population doubling times (and plating efficiency) of hybrid cell lines varied widely and representative samples were studied.

In the VA2 × mouse nervous system cell lines the number of biarmed and presumably human chromosomes was greater than the human diploid value (2n = 46) and similar to that of VA2 (Table 1). In contrast, the number of telocentric (mouse)

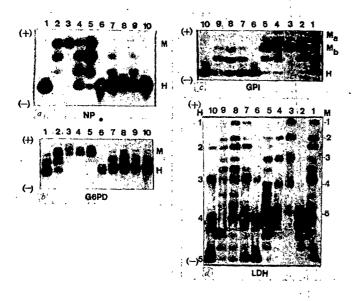


Fig. 1 Zymograms for: (a) nucleoside phosphorylase; (b) glucose 6-phosphate dehydrogenase; (c) glucose phosphate isomerase; (d) lactic acid dehydrogenase. M, mouse; H, human pattern. In (c), Ma and Mh indicate migration of mouse GPI-1A, and GPI-1B isozymes and the hybrid cell in slot 4 was derived from fusion of a VA2 cell to a mouse cell (NIH/Swiss GP) heterozygous (GPI-1AB) at this locus. In (d), 1, 2, 3, 4, 5 indicate mobility of human or mouse LDH isozyme bands alone. Cell lines were: 1, VMPTH12H; 2, VB3A; 3, C57BL/6 mouse embryo brain; 4, VMSG11; 5, VMSC24, 6, VA2; 7, VMPTH14D; 8, VMPTH12; 9, VMPOC15M; 10, VMPTH28G. Note that hybrid lines in slots 4 and 5 were preselected for retention of telocentric and loss of biarmed chromosomes, and thus represent an uncommon class while slot 2 is a VA2 × L-cell hybrid.

chromosomes was less than the mouse diploid value = 40). Because some human D and G group chromosomes in VA2 may be confused with mouse telecentric chromes, the mouse chromosomes in these hybrid cells make been overestimated. Karyological analysis by quinar mustard chromosome banding shows that translocation mouse chromosomes does not occur frequently enough interfere with these estimates (our work in preparation).

Sixty to one-hundred population doublings aftision, the hybrid strain phenotypes for 16 isozymes wertermined (Table 2, Fig. 1). The genes for the human isozs studied have been assigned to specific human chromosonncluding: 1, 2, 6, 7, 11, 12, 14, 18, 19, 20, 21 and X13. Mosthe hybrid lines continued to express all the human isozynbut failed to express certain of the mouse isozymes. A fremained balanced, expressing all the human and molisozymes tested. These balanced hybrid lines had fer than 40 telocentric chromosomes. In hybrid cell lines a reduced numbers of telocentric chromosomes, the hur isozymes exhibited equal or greater staining activity than thomologous mouse enzymes which contrasts with previousork with human x mouse hybrids (slot 9, Fig. 1). Hepolymeric isozyme bands of intermediate mobility were notor PEP A (DIP-2), cytoplasmic GOT, LDH A, MDH BPI, IPOdimeric, NP, IDH1, and G6PD in these hybrics reported for other human x mouse hybrids 13-15, which pports the homology of the markers for scoring purposes. few hybrid strains retained telocentric and lost biarmed comosomes (less than 5% of the total lines tested) and the segregated human isozymes (Table 3). In contrast, m than 50 VA2×mouse L-cell hybrids made by us ha segregated human while retaining mouse markers, suggest that the SV40 genome in VA2 is not the main determininactor in loss of mouse chromosomes.

Table 2 Expression of mouse isozymes in VA2 × mouse nervous system hybrid cells (human isozymes always expred)																
Cell line	ADA	GPI	LDHA	TRIP-1	MDHI	IPO-A	PGM2	DIP-2	GOT	HK	NP	DIP-I	MPI	ID-1	LDHIG6PD	HAT
C57BL/6 brain VA2	+	+	+	+	+	+	+	_	+	+	+	+	+	<del>- -</del>	++++	+
VMPOC15M VMPOC18R VMPSC10D VMPOC15F VMPE18F VMPOC18E VMPTH14C VMPTH12 VMPTH12 VMPTH28B VMPTH28B VMPTH28C VMPTH28G VMPTH28G VME10 VME15 VMSG4 VMSG6 VMSG6 VMSG5	+++++++++++	++++++++++	+++++++++++	++++++++++	++++++++	++++++	++++++++++++++	+++-+2+-+++++++	+++++	+++++	++++++	+++++	+++	++   +++	++++++++++++++++++++++++++++++++++++++	++++++++++

Cells were shifted from F to D medium with or without HAT supplementation depending on their ability to grow in HAT. For unexplained reasons, not all hybrid cell lines were capable of growth in DHAT after growth in FHAT (plating efficiency < 10<sup>-5</sup>) in spite of the ability of VA2 to grow well in either F or D medium. The cell lines were free of mycoplasma contamination by bacteriological analysis (dicrobiological Associates). Cells at confluence were washed three times with saline D<sub>1</sub> supplemented with 5.6 mM glucose, and 59 mM sucress sonicated at 4° C in 50 mM KPO<sub>4</sub>, pH 6.8 (total homogenate protein concentrations?, 10-20 mg ml<sup>-1</sup>) frozen on dry ice, stored under the vapour phase of a N<sub>2</sub> freezer. Brains from C57BL/6 6-week-old makes, or 16-18-d in utero embryos were processed similarly. Thawed homogenates were centrifuged (30,000g, 30 min, 4° C) and 250-500 µg of supernatant fluid protein was used per slot in a Buchler vertical starch ge electrophoresis using Electrostarch (Otto Hiller) and isozymes were scored by standard procedures 8-12: lactic dehydrogenase A and B (LIH A, LDH B); peptidase B (PEP B, mouse TRIP-1); peptidase C (PEP C, mouse DIP-1); peptidase A (PEP A, mouse DIP-2); glucose 6 phosphate dehydrogenase (GPD); indophenol oxidase dimeric (IPO-A); mannose phosphate isomerase (MPI, mouse MPI1); nucleoside phoshorylase (NP); hexokinase (HK); adenosine dearminase (ADA); cytoplasmic glutamate-oxaloacetate transaminase (GOT1, mouse GOT1; glucose phosphate isomerase (GPI); isocitrate dehydrogenase (IDH1, mouse ID-1); cytoplasmic NAD dependent malate dehydrogenase (MDH1; phosphoglucomutase 1 (PGM<sub>1</sub>, mouse PGM<sub>2</sub>). LDH B is activity of band 1 (see Fig. 1d) relative to VA2. HAT column in the tables incicates the ability of cell line to grow in HAT medium. Mouse isozyme nomenclature, when available, is used in this table. N = No activity; cash = uncertain.

Tai	Ехр	ression	of huma	ın isozy	mes in \	/A2 × r	nouse ne	ervous s	ystem hy	brid ce	lls (hu	man iso	zymes	not alv	vays exp	ressed)	
Cell line	ADA	GPI	LDHA	PEPB	MDHI	IPO-A	PGM1	PEPA	GOT1	HK	NP	PEPC	MPI	IDHI	LDHB	G6PD	HAT
VMSC24 VMSG11 VB3A VMSC14 VMSC16 VMSG2	++++	+++++++++++++++++++++++++++++++++++++++	+ - + - -	++	+ + - + - + -	+ + + -	++++++	+++ <b>ZZ</b> -	+ + - - -	+ + +	++++	+++++++++++++++++++++++++++++++++++++++	<del></del>	+ - + + -	   	+	+++++

These hydrines were preselected from over 100 VA2 × mouse nervous system hybrids for retention of telecentric and loss of biarmed chromosoma these few lines, the mouse isozymes were always expressed and the (+) and (-) symbols refer to the expression of human isozymes. V = VA2 × B82 (mouse L cell) hybrid line is presented for comparison as an example of a VA2 × mouse tissue culture line. Human isozyme nomenclature is used in this table.

Hybrid cretaining human and losing mouse chromosomes provide inflation for mouse linkage studies. Our data from VA2×mouhervous system hybrids suggested associations of mouse ismes (Tables 2 and 4). Mouse GPI, associated with mouspkage group (LG) I11, exhibited an apparent synteny wiLDH A and TRIP-1 (human nomenclature, PEP B). Inher experiments, Chinese hamster fibroblast × normal motspleen hybrids segregating mouse chromosomes and isozymhave yielded a few examples of asynteny of expression couse GPI, TRIP-1, and LDH A (in preparation). Pitfalls exi in somatic cell linkage analysis including non-randomgregation of chromosomes<sup>16</sup>. In fact, since some isozymes weexpressed more frequently than others, our data suggest nonndom segregation of mouse markers in this system. Syny data should be considered tentative and the presence or sence of specific mouse chromosomes must be demonstrateo be correlated with specific mouse isozymes.

Eight hylls between VA2 (HPRT-) and mouse nerve tissue (HPR+) that had lost telecentric chromosomes were removed fro HAT medium about 40 doublings after fusion,

1

grown in medium F for forty doublings, and then found to be unable to grow in HAT medium. When tested for G6PD, apparent synteny was noted between the ability to grow in HAT and expression of mouse G6PD. It will be interesting to determine if mouse HPRT and G6PD are correlated with the presence of the mouse X chromosome as they are with the human X chromosome<sup>13,17</sup>.

Several examples of asynteny of mouse isozyme expression, generated from the data in Table 2, are summarised in Table 4. With the exceptions of GPI, LDH A and TRIP-1, the other mouse isozymes appeared to be asyntenic with each other. Thus, these isozymes probably mark 12 of 19 mouse autosomes. One case is perplexing and emphasises the need for caution in interpreting the results. DIP-1 and IDH-1 have both been assigned to LG XIII in the mouse<sup>11</sup>. Yet, three instances of asynteny were detected in hybrid clones (Table 4). In all cases, DIP-1 was expressed while IDH-1 was not, possibly due to weak staining of mouse IDH-1.

Synteny of human PGM1 and PEP C and their correlation with the presence of chromosome 1, IDH and MDH1 with

	T	able 4 N	o. of VA	.2 × m	ouse ner	vous sys	tem hy	brid stra	ins show	ing asy	nteny fo	r mouse	isozyme	s(N =	22)	
G6PD	H 0	AT C	5PD					water to the second	7,000							
GPI	6	60	GP GP	ıτ												
LDHA	6	6	91		НА											
TRIP-1	6	6	0	9		IP-1										
ADA	· 6	6	3	3	3											
PGM2	1	1	3	3	3	AD 1		GM2								
MDH1	4	4	2	2	2	4	3		DH1							
IPO-A	2	2	2	2	2	4	3	2		D-A						1
DIP-2	5	5	5	5	5	5	4	5	3		P-2					0
GOT	. 9	9	7	7	7	9	8	7	9	8		TC				
HK	6	6	6	6	6	8	7	4	6	7	3	HK	-			
NP	9	9	7	7	7	9	8	5	7	7	4	1	NF	•		
DIP-1	12	12	9	9	9	9	8	7	4	6	2	3	4		P-1	
MPI	10	10	8	8	8	10	9	6	6	5	5	2	1	3		PI
ID-1	[1	11	11	11	11	11	10	9	9	. 8	• 4	5	6	2	3	ID-i
LDHB	≥ 1	≥11	≥8	≥8	≥8	≥8	≥9	≥10	<u>≥</u> 10	≥8	≥11	≥12	≥11	≥14	≥12	≥ 14

The data in Table 2 were collated for 22 hybrid clones segregating mouse chromosomes that arose as independent mating events or exhibited differences in their isozyme patterns. The number of cases of lack of correlation (asynteny = +, -, or -, +) between two different isozymes was tabulated. In the case of LDHB (LDH1) where mouse and human phenotypes cannot be distinguished but VA2 has low LDH1 levels only +++ or ++ activity was used as positive, and (-) used as negative. Mouse isozyme nomenclature is used in this table.

chromosome 2, and LDH B and PEP B with chromosome 12 have been reported<sup>13</sup>. VA2-mouse nervous system hybrids have yielded examples of asynteny of expression in the mouse for each of these isozyme pairs. Thus our studies suggest interspecies differences in these linkage relationships. We anticipate, however, that interspecies homology may be detected when markers short map distances apart or on the X chromosome are considered.

Jami et al.18 presented preliminary evidence of a case of retention of human and loss of mouse chromosomes in one and possibly two rare hybrids formed by fusing VA2 to mouse tissue culture L cells. In their report, however, no data were presented (such as absence of a mouse isozyme) which would imply loss of both homologues of any of the mouse chromosomes. Most of their VA2×L-cell hybrids, as well as similar hybrids produced by Weiss et al.2 retained mouse and lost human chromosomes. Our results describe a general method (fusion of freshly liberated mouse cells with an established line of human cells) for producing reverse segregant hybrids as well as evidence that such strains do lose mouse chromosomes and isozymes.

The isozymes we studied were codominantly expressed in at least some hybrids from these matings and in hybrids of the type that retain mouse and lose human chromosomes<sup>13</sup>. It is likely for most isozymes studied that chromosome segregation, and not gene regulation, is responsible for the lack of expression. In addition, molecular hybridisation studies have demonstrated that mouse nuclear DNA can be lost altogether from such hybrids4. When mouse isozymes capable of codominant expression are not detected in hybrid cells, presumably both chromosome homologues have been lost from the cell line. Miller19 has shown in man-mouse hybrids segregating human chromosomes, however, that considerable heterogeneity of human chromosome complements can exist within a hybrid line in spite of a constant mean number of human chromosomes per cell. Thus, the total number of different chromosomes represented within a line may be quite large. Similar analysis will be needed of hybrids segregating mouse chromosomes.

The basis for the novel pattern of chromosome and isozyme segregation in these hybrids is undetermined. It does not appear to be a unique property of VA2 since similar results were obtained with human cell D98AH-2 (American Type Culture Collection CCL18.3). Nor are embryonic cells unique; adult mouse bone marrow and spleen cells also give reverse segregating hybrids with VA2. The nervous system cell fused (for example neurone or glia) is not known; however, further study of differentiated functions in the hybrids may establish their parentage. Since these hybrid strains retain complete human chromosome complements, they represent new combinations of human and mouse genetic information for work such as analysis of mitochondrial DNA composition<sup>4</sup> or study of viral gene regulation 20.

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#### Activation of low molecular weight fragment of antihaemophilic factor (factor VIII) by thrombin

THE precise molecular function of plasma alaemophilic factor (AHF, factor VIII), a glycoprotein with apparent molecular weight of 2 × 105, is unknown. Recessperiments suggest that this function may be carried out by av molecular weight (LMW) fragment which can be dissociaterom plasma AHF by increased salt concentration1-3. The LW fragment retains procoagulant activity, but is not reactivn immunoprecipitation assays for AHF-related antigen4. A ih molecular weight (HMW) fragment, also identified using h salt conditions, has no procoagulant activity but retainAHF-related antigen in immunoprecipitation assays and vi Willibrand factor activity in washed platelet assays4,5. We he now found that the LMW fragment, as well as AHF8-9, isisceptible to activation by thrombin. This observation sugsts that the LMW fragment is not a product of thrombin actition of AHF and verifies the proposed role of the LMW fragent in AHF procoagulant activity.

Partially purified AHF was obtained by agaroigel filtration of an AHF concentrate (Abbott Laboratories, Sth Pasadena) using the method of van Mourik and Mochtar! AHF in the void volume fractions was concentrated by adng an equal volume of 30% polyethylene glycol (PEG); the ecipitate was separated by centrifugation at 39,000g at 20° C r 30 min and was redissolved in one-tenth the original volumin imidazole

Table 1 Thrombin activation of AHF and LM\fragments

Incubation time	AHF activity (U 1-1)						
(min)	Purified AHF*	LM\ fragments*					
0	1.0	1.0					
3	11	9.0					
8	2.2	6.4					
15	0.9	3.5					
30 70	0.6 0.3	2.5 1.6					
70	0.3	1.0					

<sup>\*</sup> Material was diluted in barbital-buffered salir4 so that each contained 1 U ml<sup>-1</sup> AHF activity. To each was aded 1/20 volume phospholipid (final concentration 0.05 mg ml<sup>-1</sup>) ad 1/20 volume purified thrombin (final concentration 0.035 U ml<sup>-1</sup>). The mixture was incubated for 3 min at 37° C and then placed in at ice water bath. Samples were removed for AHF assays<sup>12</sup> at the specified times. AHF activity before addition of thrombin is identified a 0 min. Control activity before addition of thrombin is identified a 0 min. Control assays were carried out in which thrombin and piospholipid were added to buffered saline. This mixture had an appaent AHF activity less than 0,001 U ml-1 and was not different from the blank time.

_	rapie 2	ombin activation of AH	F and HMW fragments
	Incubati	me AHF 2	activity (U ml <sup>-1</sup> )
	(mi	Purified AHF*	* HMW fragments*
	(	0.2	0.001
	3	1.6	0.02
	8	0.3	0.001
	15	0.2	0.001

\* Materia ted in barbital-buffered saline so that each contained 1 U of AHF-related antigen by radioimmunoassay 13. Assay edure was otherwise as described for Table 1.

(0.05 M)-bled saline (0.14 M) pH 6.8. This sample was dissociated tel filtration in 0.24 M CaCl, and 0.05 M imidazole, pH 6 sing a Bio-Gel A-15m (BioRad) column of  $1.6 \times 30$  cnth a flow rate of 20 ml h<sup>-1</sup> at room temperature<sup>4</sup>. HMW and W fractions were pooled separately and concentrated with equal volume of 30% PEG as indicated above; the precipit were each dissolved in one-tenth the original pool volumin imidazole-buffered saline. Bovine thrombin (Parke-Daylas purified by Lundblad's method<sup>11</sup>. The specific activity of thrombin was 1,333 U mg<sup>-1</sup> as determined by a reference cu prepared with standard thrombin supplied by Dr David Ason (Bureau of Biologics Standards, Bethesda).

Thrombirtivation of AHF and the LMW and HMW fragments was amplished as described in Table 1. Activation of AHF and IW fragment was maximal after 3 min, the procoagularactivity increasing tenfold over the starting activity (Tal 1). Subsequent inactivation of procoagulant activity was's marked for the LMW fragment than for purified AHF. Rults were similar in three experiments.

Low levels AHF procoagulant activity were also detected 3 min after tombin activation of the HMW fragment. Table 2 presents rest from a typical experiment; shortening of the clotting timevas observed in five instances. Thrombin activation of the MW fragment may indicate incomplete dissociation of the MW from the HMW fragment. Alternatively, masked pot(ial procoagulant activity may be exposed by alteration of inactive HMW fragment by thrombin.

Thus AH ragments produced by dissociation in high ionic strength buffs retain the capacity for thrombin activation and inactivation. The demonstration that thrombin activates the LMW fragmit provides additional evidence that this part of the AHF coolex is important for AHF procoagulant activity as measured vitro. A corresponding physiological role for LMW AHFragments is suggested by the detection of low molecular wight procoagulant activity after transfusion in patients withon Willebrand's disease14.

The molellar basis for thrombin activation of AHF and the LM7 fragment derived from AHF—is unknown. As the LMW frement retains a capacity for thrombin activation, it is unlikely be a product of thrombin action on intact AHF. The absenceof detectable proteolytic products when highly purified AHFis activated by thrombin<sup>15,16</sup> supports this conclusion. Thromin activation of AHF is an important but poorly understood penomenon; studies with LMW fragments will permit more robing analysis of this interaction.

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#### Myosin linked calcium regulation in vertebrate smooth muscle

CONTRACTILITY resulting from the interaction of actin and myosin filaments is in general controlled by the concentration of calcium ions. The regulation of this interaction by Ca ions can, in principle, be exercised at the level either of the actin or of the myosin filaments and both these types of regulatory system are found in nature. In vertebrate skeletal muscle troponin is the Ca receptor and is attached to the actin filament. The action of troponin is inhibitory: when no Ca ions are bound to it the interaction between actin and myosin is prevented1-4. This inhibition is relieved and interaction occurs at calcium concentrations sufficient for the Ca binding sites on the molecule to be occupied. The second type of regulatory system was discovered in molluscan muscles and is present in other invertebrate systems which lack troponin5,8. In some cases both types of regulation seem to be present in the same muscle<sup>9.7</sup>. Here I present evidence which shows that such a myosin-linked regulatory system is not confined to invertebrates but is also present in smooth muscles of higher vertebrates. A more detailed description of the regulatory system in this muscle type is presented elsewhere8,8.

Rabbit actin filaments without regulatory proteins attached have been used in a diagnostic test for the presence of myosinlinked Ca regulatory systems6. The principle underlying this competitive actin-binding assay is that if an actomyosin contains Ca-regulated myosin filaments, the ATPase activity of the preparation will not be stimulated when unregulated actin filaments are added; Ca ions will still be required for activation. This is the case when purified rabbit actin filaments<sup>10</sup> are added to smooth muscle (chicken gizzard) actomyosin (Table 1). The ATPase activity shows the identical sensitivity to Ca both in the presence and absence of a molar excess of actin molecules. Other experiments have established that actin and myosin of smooth muscle actomyosin dissociate under the conditions used in this experiment and thus the myosin molecules were free to complex with the added skeletal muscle actin. The turbid smooth muscle actomyosin undergoes a dramatic clearing reaction when ATP is added, coincident with the dissociation of actin and myosin. Observation of a reaction mixture indicated that no incipient precipitation occurred when the actin filaments were added and therefore that the actomyosin remained dissociated. Since Ca is required for stimulation of the ATPase activity in the presence of unregulated skeletal actin, the absence of Ca must have a direct effect on the myosin which prevents its interaction with actin. Precisely how Ca determines whether or not the myosin will combine

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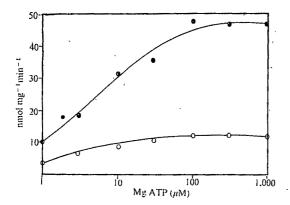


Fig. 1 The rate of ATPase activity was determined using a creatine kinase ATP regenerating system<sup>3</sup>. The reaction medium contained 60 mM KCl, 20 mM imidazole (pH 7.0), 5 mM creatine phosphate, 0.5 mg ml<sup>-1</sup> creatine kinase and 5.3 mg ml<sup>-1</sup> GAM, 1 mM MgCl<sub>2</sub>, 0.5 mM cysteine, and either 2mM EGTA or 100 µm CaCl<sub>2</sub>. The MgATP concentration indicated is the added concentration, the myosin concentration is about 7.3 µM. ATP was added as the Mg chelate, and therefore the Mg<sup>2+</sup> concentration, was 1 mM throughout the experiment. The reaction time varied between 3 and 30 min at 25° C. ●, +Ca; ○, -Ca.

with actin is not understood; it could be through an electrostatic effect or through a conformational change in the molecule.

In vertebrate smooth muscle preparations, no troponin-like components can be identified by SDS gel electrophoresis8,9, But to demonstrate the absence of troponin by another, independent method, a functional test, which is essentially the converse of the experiment above, was carried out. For this experiment rabbit heavy meromyosin (HMM) which itself is not calcium responsive was mixed with the smooth muscle actomyosin (Table 1). If no Ca regulatory system is present on the actin filaments the HMM ATPase activity would be expected to be unrestrained at low Ca concentrations; otherwise it would be inhibited. As expected of an actomyosin without Ca-regulated actin filaments, the ATPase activity after HMM addition is less sensitive to Ca than the control.

Subtracting the contribution of the smooth muscle actomyosin from the total activity shows that the actin stimulation of the HMM ATPase activity is actually depressed in 100  $\mu M$  Ca. Since the smooth muscle actomyosin superprecipitates in the presence of 100 µM Ca, this depression can be accounted for by the complexing of the smooth muscle

Table 1 Locus of the calcium regulatory system in smooth muscle actomyosin

Components	nmol m		Ca sensitivity (Activity 10 <sup>-4</sup> M Ca)
	10 <sup>-9</sup> M Ca <sup>2+</sup>	10-4M Ca <sup>2+</sup>	(Activity 10 <sup>-9</sup> M Ca)
(a) Gizzard actomyosin (GAM) (1.3 mg ml <sup>-1</sup> )	4.1	21.7	5.3
(b) GAM (1.3 mg ml <sup>-1</sup> )+ rabbit actin (1 mg ml <sup>-1</sup> )	4.4	23.2	5.3
(c) GAM (1.3 mg ml <sup>-1</sup> )+ rabbit actin (1 mg ml <sup>-1</sup> ) +chicken breast	4.4	23.1	5.3
tropomyosin (0.2 mg m)			
(d) GAM (1 mg ml <sup>-1</sup> )+ Rabbit HMM (0.5 mg m	29.1	39.7	1.4
(e)(d)-(a)	25.0	18.0	; <b>0.7</b>

The weight ratio of tropomyosin:actin: myosin in the smooth muscle actomyosin is approximately 0.3:1.0:2.4 (ref. 8). The ATPase activity was determined by phosphate liberation after mixing gently for 10 min at 25° C in a medium containing 1 mM MgATP, 40 mM KCl, 20 mM imidazole (pH 7.0), and either 1 mM EGTA or 100  $\mu$ M CaCl<sub>2</sub>. The rabbit actin filaments and the chicken breast tropomyosin were determined to be free of contaminating proteins by SDS gel electrophoresis analysis. In (D) the rate of HMM ATP hydrolysis in the absence of actin (3.4 nmol ml-1 min-1) has been subtracted and thus the values given are actin-stimulated activities.

myosin with a fraction of the actin molecules, thereducing the number available to stimulate the HMM AT activity.

Another distinguishing feature of actin-linked ared with myosin-linked regulatory systems is their respon changes in MgATP concentrations11. Relaxation of skl muscle systems does not occur at low MgATP concentratiwhere the myosin active sites are not saturated with sate. The raised ATPase activity3,4,12 and persistence offtraction13 which occur under these conditions is indirectlyjed by the substrate-free myosin molecules. They form rigiomplexes with actin and nullify the inhibitory influenf Ca-free troponin3.4. If myosin is subject to Ca regulation, sa biphasic response-stimulation at low and inhibition ath MgATP concentrations-is not expected and is not foun myosinlinked regulatory systems11.

Smooth muscle actomyosin responds in a manypical of a myosin-linked regulatory system; the response it biphasic. ATPase activity at the low Ca concentration incremonotonically, and is Ca sensitive (that is, relaxationurs, at all MgATP concentrations even when the numbof myosin molecules exceeds that of substrate molecules) (11). Similar results have been obtained in superprecipitation periments with actomyosin and in ATPase activity meaments and superprecipitation experiments with smooth mulmyofibrils.

My results are consonant with those of Filoal.14 where tension measurements were performed at diffit MgATP concentrations on guinea pig taenia coli. In thetudy, however, the concentration of MgATP was chan by adding various amounts of Mg to a constant amount ATP. The interpretation of the data obtained under thesonditions is not straightforward because the ATP anion has daxing effect on smooth muscle8,8,15, and also because Mg ionave specific effects on smooth muscle contractile apparatus8. he results of these workers indicate nevertheless, that myosiegulation is not restricted to chicken gizzards.

The above results indicate that the myosin ked calcium regulation found in primitive organisms has en retained during the evolution of higher vertebrates. Bothpes of regulatory system have evolved in parallel and are preit in different types of muscle in the same animal. In view ohe similarity between the myosin of smooth muscle and that fri non-muscle sources, especially with respect to their immungical characteristics16,17, and with respect to the light chaisize8,9,18-21 it is not unlikely that a similar mode of Ca regulon is present in these other systems as well. If this can be subintiated, then the study of smooth muscle, which is more reily available, will throw light on these other less well deed forms of contractility.

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#### Calcium hophore A23187 potentiate witch and intracellular calcium release iningle muscle fibres

THE potentian by drugs of the twitch force in skeletal muscle has generallyeen related to a prolongation of the 'active state' in thexcitation-contraction coupling mechanisms1.2. This can, hower, only be partially correct since in another type of potention elicited by repetitive stimulation (staircase phenomenon the 'active state' was found to be intensified but not proliged3. This view that the 'active state' is not maximum in lingle twitch and that it can indeed be intensified in twitch poteiation was criticised but subsequently received support from everal studies 5,6. On the other hand it is well known that tivation of muscle contraction involves rapid changes of e intracellular calcium concentration which result from ceium release and uptake processes in the sarcoplasmic reticum (SR)<sup>7,8</sup>. The discovery of specific ionophores (antibiotics abwing cations to cross biological membranes by way of a frly electroneutral mechanism) which selectively increase the jembrane permeability to calcium9-11 offers a new tool following these intramuscular processes. For example, ionohore X537A was found to alter the contractile responses an resting tension of strips of rat diaphragm, an effect which as ascribed primarily to an increased calcium influx across he muscle fibre membrane<sup>12</sup>. On the other hand the ionophore X537A and A23187 tested on SR vesicles and mitochondriaisolated by ultracentrifugation were found to induce a release of stored calcium10.12.13.

The present nvestigations indicate that the calcium ionophore A23187 (Eli killy) strongly potentiates the twitch force of single muscle ibres and acts directly on the process of calcium release from the SR, even in these intact fibres. The experiments were performed on muscle fibres isolated from the depressor muscles of the barnacle, Balanus nubilus, in conjunction with two different procedures as described recently14: (a) the intracellular concentration of calcium ions at rest and during activation was studied in large fibres 1.0 to 2.0 mm diameter which had ben micro-injected with the calcium-sensitive bioluminescent protein aequorin15 and placed in a light-tight chamber in flont of an EMI 9635 photomultiplier tube; (b) the calcium putflux across the outer membrane was estimated in single barnacle fibres loaded by an intracellular injection of 0.1 to 0.2  $\mu l^{1.45}$ Ca in Tris buffer at pH 7.2 (ref. 16). The calcium ionophore A23187 was dissolved at 10<sup>-3</sup> M in ethanol

which was added to artificial seawater (ASW) to obtain a final concentration chosen from  $10^{-7}$  to  $10^{-5}$  M.

Figure 1 illustrates the resting calcium outflux from a single barnacle muscle fibre equilibrated in ASW, two hours after <sup>45</sup>Ca loading. Ethanol 1% added to the external medium was found to increase the resting outflux by 90% above baseline. At these concentrations the ethanol effect reached its maximum in about 30 min. After this control test A23187 10-5 M in ASW (thus containing also 1% ethanol) was substituted externally: the resting calcium outflux increased markedly to reach a new level, 450% above the extrapolated baseline. The maximum increase of calcium outflux induced by A23187 was usually observed after 30 to 50 min in 10<sup>-5</sup> M A23187. The increase of 45Ca outflux was recorded in all experiments but its amplitude depended upon the ionophore concentration; the recovery on return to ASW was influenced both by the duration of exposure and by the concentration used. The findings cannot be explained by an increased exchange diffusion across the outer fibre membrane because similar A23187induced augmentations of 45Ca outflux were recorded in experiments with 0 Ca in the external solution. These data suggest that the intracellular calcium storage sites are involved.

This was tested by recording the rate of light emission of a barnacle fibre micro-injected with aequorin15,14. The fibre was stimulated at intervals of more than 30 s through an intracellular silver wire with square electrical pulses of 75 or 150 ms duration of various intensities. The membrane potential was monitored with another intracellular platinum electrode connected to a

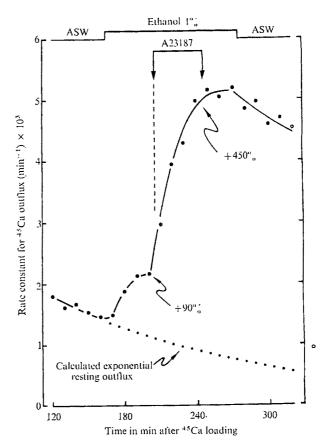
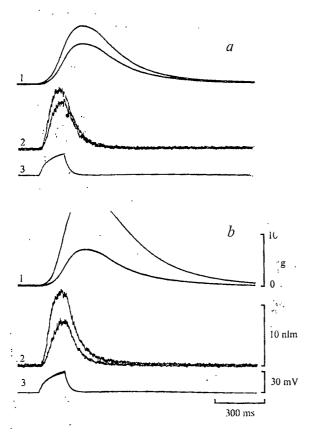


Fig. 1 Effect of A23187 10<sup>-5</sup> M on the <sup>45</sup>Ca outflux from a single barnacle muscle fibre. The exponential resting outflux curve  $(y=ae^{bx}; a=57.09 \text{ and } b=-0.01;$  coefficient of determination  $r^2=0.7831$ ) was calculated from the experimental data recorded after 2 h equilibration of the fibre in ASW. The ionophore solvent, ethanol, modified the resting outflux: 90% above baseline after 30 min. This was followed by a marked increase of the outflux (450% in 40 min) when A23187 was added to the external medium. Only a moderate recovery was observed when this fibre was transferred back to ASW. Fibre diameter: 1.0 mm; mean intracellular resting potential: -48 mV; temperature of mean intracellular resting potential: -48 mV; temperature of solutions: 22° C.



Result of applying a single electrical stimulation of 150 ms duration to a barnacle muscle fibre injected with aequorin. Two traces are superimposed in each record: one before the application of A23187 serving as control and the other after the ionophore had been present for 10 min. Trace 1, isometric tension; trace 2, calcium mediated light emission; trace 3, membrane response. The slight decrease observed in trace b,3 was recorded in presence of A23187. Fibre diameter: 1.9 mm; mean intracellular resting potential: -54 mV; temperature of solutions: 23° C. a, A23187 (10<sup>-6</sup> M); b, A23187 (10<sup>-5</sup> M).

high input impedance amplifier<sup>15,14</sup>. Figure 2 illustrates typical results obtained on the same muscle fibre in the presence of two different concentrations of A23187, under identical stimulation conditions. Two traces are superimposed in each record, one before the application of A23187 serving as control and the other after the calcium ionophore had been present in the external medium for 10 min.

The membrane depolarisations actually elicited by the constant intensity stimulus (record 3) were stable in the control and test trials which indicates that any difference in the force output (record 1) or in the calcium transient (record 2) must result from genuine changes in the mechano-chemical activation processes (compare ref. 15). No regenerative action potential was elicited by the stimuli as shown by the intracellular record 3. In the presence of 10<sup>-8</sup> M A23187 the mechanical force output (record 1) increased by 42% but it was not prolonged (Fig. 2a). The size of the calcium transient (record 2) increased by 28% in Fig. 2a and its rate of rise was much accelerated so that the peak was reached somewhat earlier. The duration of the calcium transient was not significantly prolonged and the time from peak to half decay was indeed slightly reduced. A concentration of 10<sup>-5</sup> M A23187 was tested in Fig. 2b. After 10 min the force was considerably increased to about 3 times control. The rate of force development was accelerated. The calcium transient was increased by 70%, its rate of rise was accelerated and the time from peak to half decay was about the same as that of control. These results were regularly obtained in 5 muscle fibres from 4 different barnacles, each one being tested with different combinations of stimulus parameters and of ionophore concentration from  $10^{-7}$  to  $10^{-5}$  M. The above modifications returned to

control values when the fibre was transferred to without ionophore in a time variable from 10 to 20 min.

The above results suggest that when the calciunophore A23187 10<sup>-7</sup> to 10<sup>-5</sup> M is added to the fluid bay a single giant muscle fibre, its action involves not one outer membrane (Fig. 1) but also the intracellular cal storage sites. The latter effect was very strong and a standapolarisation step imposed to the outer membrane evokearger and faster release of calcium which in turn elicited ath potentiated twitch (Fig. 2). The resting light emissivas only slightly increased when the potentiation was ided. The A23187 potentiation which occurs without alignificant prolongation of the mechano-chemical events pres a formal resemblance to the staircase potentiation3 which onsider to involve primarily the calcium release from SR. effect of A23187 has a rather rapid onset and large polations of the contraction can be recorded after only 1 or 2 m exposure to A23187. The effect is reversible and does apparently disturb the sequence of events involving calciumuestration by SR during the relaxation of the muscle fibre. records of Fig. 2 make it clear that the calcium ionophorethese concentrations provides a useful tool to manipulattracellular processes of excitation-contraction coupling skeletal muscle fibres directly.

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#### Precocious development of detoxicating enzymes following pituitary graft

In vertebrates many biologically-active enogenous compounds, (such as bilirubin, steroid hormones and the catecholamines) and an enormous range of drugs, carinogens, pesticides, industrial pollutants or their metabolitesare made more polar and excreted by conjugation with glicuronic acid1. This is the principal route of detoxication but only one mechanism for the biosynthesis of such 'glucuroniles' is known:

Table 1 En embryo-liver UDP glucuronyltransferase activity

or gratting out	adult tissues on to chorioaliantoic memorane
Tissue	nmol o-aminophenyl glucuronide formed per mg protein h <sup>-1</sup>
None	Trimed but will be true a
(sham-opli)	0.6, 0
Adrenal gla	0, 0
Testis	0, 0
Skeletal mu	0.6, 0
Liver	1.0, 0
Brain	1.1, 0.5
Pituitary gla	6.5, 6.3, 6.5
1	

Tissue frd week White Leghorn cockerel was placed on the chorioallanthembrane of 12 d embryos, and GT activity assayed in embryo I on day 15. Results are from separate experiments. Weights of ther tissues were 1-3 times the weight of the single pituitary gland grafted.

glucuronyl Insfer by UDP glucuronyltransferase (GT) (EC 2.4.1.1) the accepting compound<sup>1</sup>.

GT occumily at very low levels in the embryo or foetus<sup>1</sup>; it reaches addevels around birth, the rate depending on substrate and cies. Because much drug toxicity and jaundice in the newn arises from this temporarily defective GT activity, thearch for factors controlling induction of the enzyme has n extensive.

GT activ can be increased in newborn2 or term-foetal liver (B. Buell and G. J. D., unpublished) by phenobarbital, and this druan induce GT in chick embryo liver in ovo3 or in culture4. Thendogenous inducers or inducing mechanisms, however, haremained unknown.

We reported the precocious increase of GT from virtually zero to adulvels in the livers of chick embryos in ovo, following the grafg of chicken pituitary gland on to the chorioallantoic merane. This observation should enable the identification of tlendogenous inducing mechanism of this major detoxicating zyme. We have found that pituitary grafts also allow premarely adult expression of one of the microsomal mixed-functi oxidases (EC 1.14.1.1.), enzymes which hydroxylate a molule before glucuronidation<sup>5</sup> and which are therefore also imptant in today's environment.

GT activitis detectable in chick embryo liver at 8 d but remains verlow until hatching at 21 d, when it increases dramatically adult levels. Because the culture of isolated liver out of the embryonic environment allows precocious induction of T<sup>7</sup>, an in ovo 'repression' of the enzyme has been postulated. e were unable to overcome this repression by varying O2 nd CO2 tensions, by incubating embryo and membranes an open dish, by hypophysectomising the embryo at 3h (ref. 8) or by injecting known hormones from mammalian frenal cortex, thyroid and pancreas.

The pituity begins to secrete in foetuses just before the emergence of the late-foetal cluster of enzymes, amongst which is GT and an injection of pituitary extract brought about the precociot appearance of xanthine oxidase in chick embryo liver10. We herefore attempted to evoke GT by injecting various compercial extracts or freshly-made homogenates of mammalian or chicken pituitary glands into the egg. Our results were iconclusive, and we decided to graft adult chicken

Table 2 De lopment of UDP glucuronyltransferase activity in liver of embryos exposed to pituitary grafts

	- or willow you emposed to pitt	arran, grants
Day 14 15	nmol <i>o</i> -aminophenyl per mg p Test 0.7, 0.5, 1.1, 1.3 6.5, 6.3, 6.5 32.9±15.1 (7)	glucuronide formed rotein $h^{-1}$ Control $0.6\pm0.4$ (5) $0.6\pm0.4$ (5) $0.6\pm0.2$ (5)

Pituitary gland from a 6 week cockerel was placed on chorioallantoic memirane of 12 d embryo and GT assayed1 in embryo liver on days roted. Results are of individual experiments or s.e.m. with number of experiments in parentheses. 'Adult range' for this batch of 6 week cockerels was 30-60 of above units. glands on to the chorioallantoic membrane to allow a more physiological uptake of possibly unknown factors.

Pituitaries were therefore taken from 6-8 week old hens or cockerels and grafted, one per egg, on to the chorioallantoic membrane of 12 d eggs. Other eggs received grafts of other tissues, control eggs were sham operated and all eggs were then returned to incubation. Embryo livers were examined on subsequent days. By the third day, embryo-liver GT activity had risen markedly in those eggs which had received grafts of pituitary gland; adult brain, adrenal, testis, liver and muscle grafted on to the membrane did not significantly alter the normal negligible embryo-liver levels of the enzyme (Table 1).

The rise in GT activity was time-dependent. It was not notable until day 3 after grafting and increased during days 3 and 4. It reached the adult range on day 4 (Table 2).

Activity of the mixed-function oxidase, aniline hydroxylase11, was also increased by pituitary-grafting; it rose threefold, reaching adult level. No rise was observed for either enzyme in embryo livers under 10 d old at the time of grafting and examined up to 4 d later.

Table 3 Age of pituitary gland donors and effect on embryo-liver UDP glucuronyltransferase

Donor .	nmol o-aminophenyl glucuronide formed per mg protein h-1
No donor (control) 15 d embryo 18 d embryo 20 d embryo (not hatching) 20 d embryo (hatching) 1 d chick 2 d chick 6 week cockerel	$0.6\pm0.2$ (5) $0.9\pm0.3$ (5); $18.2*$ $1.8\pm0.6$ (5); $11.2*$ 1.8 26.5, $26.41.5$ , $4.8$ , $6.312.932.9\pm15.1 (7)$

One pituitary gland from the donor was placed on the chorioallantoic membrane of a 12 d embryo, and liver GT assayed1 on day 16. Results expressed as in Table 2. \*Two glands placed on membrane:

Pituitaries from 2 d-old chicks proved almost as effective as those from adults. Younger pituitaries, which were of approximately similar weight from 2 d chicks down to 8 d embryos, appeared progressively less effective except from those birds actively hatching (Table 3). Doubling the amount of embryo gland grafted evoked a marked response, however (Table 3), suggesting a threshold phenomenon.

The stimulatory effect is produced by the cephalic region of the anterior pituitary; the caudal region has negligible effect when grafted. Partly-purified preparations of hormones found in the cephalic region12 proved ineffective when applied to the membrane at various time intervals. Pituitaries from mouse and rat have so far also failed to increase embryo-liver GT, although the grafts were apparently accepted.

The cephalic region of the chick anterior pituitary gland therefore secretes a factor or factors which in the intact embryo in ovo causes precocious development of hepatic GT and aniline hydroxylase. Embryos over 9 d old are competent to respond to this factor, production of which seems enhanced by the process of hatching. The nature of the factor is now being determined and its role in the natural development of these detoxicating enzymes is being investigated.

We thank the SRC for a studentship to G.J.W., the MRC and SRC for grants, Dr T. W. Betz and Dr C. G. Scanes for valuable advice, and Eastwood Hatcheries, Fife, for supplying eggs and birds. The partly-purified hormone preparations were a gift from Dr Scanes.

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## Transcription, translation and maturation of succinate dehydrogenase during cell cycle

A stepwise accumulation of individual enzyme activities is a common feature of the eukaryotic cell cycle1. Two major hypotheses consider that the temporal restriction is produced by oscillatory repression or by linear reading of genes along the chromosome<sup>2,3</sup>. Both hypotheses, in their present form, assume that increase in enzyme activity is concurrent with transcription and translation. We have made use of the high degree of synchrony obtainable with Chlorella fusca var. vacuolata 211-8p, grown under intermittent illumination<sup>4,5</sup>, to study the duration of transcription and translation for succinate dehydrogenase by applying inhibitors during the period of its stepwise increase in activity. We report that the periods of transcription, of translation and of increase in activity are only partially concurrent.

Cycloheximide specifically inhibits protein synthesis of this strain of Chlorella<sup>6</sup> and its addition at 15 h in the 24 h cycle completely prevents the stepwise accumulation of succinate dehydrogenase (Fig. 1a) which would normally begin then?. It is striking that addition of the inhibitor less than 0.1 cycles later, at 17 h, when only a third of the full increase in activity has occurred, is unable to prevent the full subsequent accumulation of enzyme activity. Measurement of phenylalanine incorporation into protein, however, shows that cycloheximide retains its ability to inhibit protein synthesis even when the increase in succinate dehydrogenase activity is immune to its presence (Fig. 1b). The inhibition of protein synthesis is not an artefact caused by loss of labelled amino acid from the cells, as whole-cell counts show retention of the pool during exposure to cycloheximide (Fig. 1c). Therefore the accumulation of succinate dehydrogenase activity rapidly enters a phase in which it is not dependent upon continued protein synthesis by the cytoplasmic ribosomes. As succinate dehydrogenase is an enzyme of the inner mitochondrial membrane8.11, however, and as much of this membrane is synthesised within the organelle11,12, we investigated the possibility that the period when enzyme activity accumulates without cytoplasmic protein synthesis is one in which proteins must be synthesised by the mitochondrial ribosomes to allow the enzyme to develop into its catalytically active form. Chloramphenicol was shown to be an effective inhibitor of mitochondrial protein synthesis during this period of the cycle because it prevented the accumulation of cytochrome oxidase (Fig. 2a), an enzyme known from very diverse sources to contain some proteins of mitochondrial  $origin^{12-14}$ . The presence of chloramphenicol from as early as 11 h was, however, not at all inhibitory to the accumulation of succinate dehydrogenase (Fig. 2b). Similarly, lincomycin at 0.2 mg ml-1 inhibited cytochrome oxidase but not succinate dehydrogenase synthesis. Therefore that period of the increase in enzyme activity after 17 h, which does not require con-

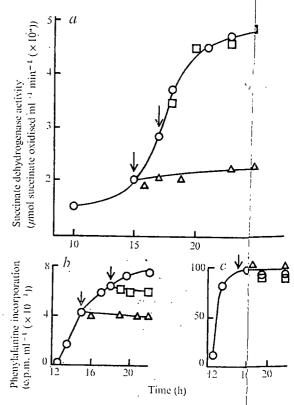


Fig. 1 The effect of cycloheximide, a, on the accilation of succinate dehydrogenase activity, b, the incoration of phenylalanine into protein and, c, the uptake of pylalanine. Cells were synchronised and held at a density of 5 × ml-1 under a regime of 15 h light and 9 h dark which wanaintained during the experimental period. Cycloheximides, $\square$ ) was applied at 2.5  $\mu g$  ml $^{-1}$  (arrows), and  $^{14}\text{C-r-}3$ -phenanine (U) was added at  $10^{-3}$  M and 25  $\mu\text{C}$  immol $^{-1}$ . The cultures sampled and assayed for succinate dehydrogenase activity described previously with the modification that for 10 min the 15 min in vitro succinate activation<sup>8</sup> period, the enzyme vincubated with 0.15 M succinate, pH 7.4, before addition the assay reagents. For estimation of phenylalanine incorpation into protein, the following procedure was necessary completely leach free phenylalanine through the sporopollenirall<sup>9,10</sup>. Five ml of culture was mixed with 15 ml of ethanol 1 stored at -15° C. The cells were pelleted, washed in cold gith medium containing  $5 \times 10^{-8}$  M cold phenylalanine and eacted with ethanol at  $80^{\circ}$  C for 3 min and ether at  $60^{\circ}$  C fd min. The protein was dissolved in 0.2 ml of 8 M urea contain;  $5 \times 10^{-3}$  M phenylalanine and 15 mg ml<sup>-1</sup> bovine serum albumind was then precipitated with cold 5% trichloroacetic acid, wasd with cold water, extracted in 0.5 M H<sub>2</sub>SO<sub>4</sub> at 100° C for h, washed briefly with hot water and dissolved in 0.1 ml of M NaOH. For counting, the alkali was neutralised with 0.ml of 1 M phosphate buffer, pH 6.8, and 0.1 ml of 10 M H(and 0.2 ml samples were mixed with 0.8 ml H<sub>2</sub>O and 10 ml scillant, which contained 50 ml Triton X-100 and 0.7 g butyl-PB per 100 ml toluene. For measurement of phenylalanine uptake mi samples of culture were passed through a membrane filternd the cells washed three times with cold growth medium the dried overnight at 105° C and counted with 3 ml of scintillai O, Control

current cytoplasmic protein synthesis (Fig. a), does not depend on mitochondrial protein synthesis either

The increase in activity cannot be attributed a change in the substrate affinity of the enzyme, for we shall how elsewhere that the  $K_m$  values for succinate and phenazinemethosulphate do not change significantly. Neither does the accessibility of the enzyme in cell extracts vary, because the mitocondrion forms a single reticulum15 which is always completed disrupted by the force necessary to break the sporopolinin-containing cell wall. Instead there seems to be a real increae in the number of catalytically active enzyme molecules betwen 17 and 24 h, due to the maturation of enzyme protein mide between 15 and 17 h.

To investigate the relationship between transription and the

2 h periodranslation we have employed 6-methylpurine. This analophibits RNA synthesis both in higher plants 18-18 and in this of Chlorella, where its effect is specifically to inhibit funal RNA synthesis by competition with adenine19. When 6-mpurine is added at 12 h the subsequent synthesis of nate dehydrogenase is completely inhibited (Fig. 3a), le is no evidence, therefore, of a persistent mRNA forenzyme throughout the cell cycle. When added at 18 h, aftenslation has ceased, the inhibitor had no effect (not showmere is, however, evidence that the newly transcribed mR is not immediately translated as, although translation does begin until 15 h (Fig. 1a), the addition of 6-methylpulto a different sample from the same culture at 15 h reveals the inhibition of RNA synthesis is only able to inhibit halfihe subsequent enzyme accumulation (Fig. 3a). Thus, aboulf of the necessary mRNA may have already been transcil at the time of initiation of translation.

This conon is only valid if 6-methylpurine is able to cause immediate bition of functional RNA synthesis: therefore its ability tompete with adenosine for incorporation into RNA was itigated at this time in the cycle. Although the analogue cotted with adenosine for uptake, there was no loss of labeladenosine from the intracellular pool (Fig. 3b) but there wan immediate 65% displacement of adenosine from incorption into RNA (Fig. 3c). Purified tritiated 6-methylpurins itself incorporated into all sizes of RNA separated by electrophoresis (C. A. Lambe and P.C.L.J., unpublished

The evide obtained by use of 6-methylpurine therefore suggests theesence of some mRNA for succinate dehydrogenase beforanslation begins. Yet there is no evidence of mRNA befol 2 h in the cycle (Fig. 3a) and so the maximum period durin/hich mRNA may be present, before translation begins at 15 is less than 0.15 cycles. Therefore, the time of succinate delrogenase synthesis is closely determined by the time of transportion. This is the first time that transcription has been shot to have a role in determining the time of syn-

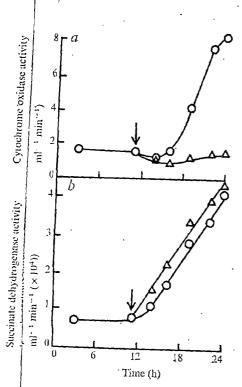


Fig. 2 The effect of chloramphenicol on, a, the synthesis of cytochrome bxidase and, b, succinate dehydrogenase. D-threo-chloramphenicol ( $\triangle$ ) was applied at 0.8 mg per ml at the times arrowed, and the cells were cultured as in Fig. 1. The culture was sampled and assayed for cytochrome oxidase as described previously and for succinate dehydrogenase as in Fig. 1. O, Control curve,

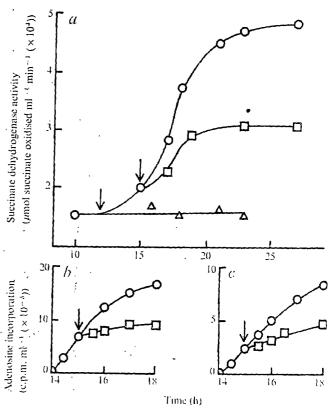


Fig. 3 The effect of 6-methylpurine on, a, the synthesis of succinate dehydrogenase, b, the uptake of adenosine and, c, the incorporation of adenosine into nucleic acid. The cells were grown as described in Fig. 1 and 6-methylpurine (Δ, ) was added (arrows) at 1×10<sup>-8</sup> M. <sup>14</sup>C-adenosine (U) was supplied at 1.25×10<sup>-6</sup> M and 26 mCi mmol<sup>-1</sup>. For measurement of total cellular uptake, 5 ml of culture was filtered through a double thickness of Whatman GF/A glass fibre filter and washed twice with cold growth medium containing 1.25×10<sup>-6</sup> M cold adenosine, then dried at 75° C overnight and covered with scintillant as in Fig. 1. For estimation of adenosine incorporation into nucleic acid, 5 ml samples of culture were made up to 5% perchloric acid and stored at 0° C. The cells were filtered, washed with 5% cold perchloric acid dried at 105° C and counted in the same way. O, Control curve.

thesis of a stepwise accumulated enzyme and there are few other investigations of the role of transcription in determining any pattern of synthesis in the cell cycle. In the thermophilic strain of *Chlorella* the glutamine analogue azaserine immediately inhibits synthesis of two autoregulated enzymes <sup>20,21</sup> and 6-methylpurine prevents the increase in glucose-6-phosphate dehydrogenase activity which occurs in excised artichoke tissue<sup>18</sup>.

There is therefore some support for the conclusion that transcriptional regulation can govern the pattern of enzyme synthesis in the cell cycle, but the delay observed here before translation of the mRNA might indicate that post-transcriptional regulation also operates. Control at this level is believed to govern isocitrate lyase synthesis under catabolite repression22 and nitrate reductase synthesis under ammonia repression<sup>5</sup> in this strain of Chlorella. Post-transcriptional controls may also be involved in the synthesis of tyrosine aminotransferase in hepatoma cells 23,24 and in the synthesis of some proteins 25-27 in other developmental situations analogous28 to progress through the cell cycle. At least some of the pre-formed mRNA, however, will be mRNA undergoing the normal procedure of processing and export from the nucleus. We therefore believe that the period when mRNA for succinate dehydrogenase is available in the cytoplasm but remains untranslated, can only be brief in relation to the cell cycle.

The enzyme is not matured into a catalytically active form before incorporation into the mitochondrial membrane, as no significant activity can be detected in the soluble fraction,

after centrifugation for 30 min at 100,000g, at any time during the phase of increasing activity. This raises the possibility that the maturation process depends on the rate-limiting synthesis of new inner mitochondrial membrane to accommodate the enzyme. In fact, a burst of mitochondrial membrane synthesis at just this stage of the cycle, after S phase, is predicted by a recent hypothesis 29,30.

Stereology has revealed that there is a continuous increase in mitochondrial volume during the cycle5.15, and furthermore we have found that the presence of chloramphenicol from 11 h in the cycle, which would be expected to block inner membrane synthesis 11,12,14, still allows the formation of fully particulate enzyme. Maturation is thus not limited by the availability of membrane to accommodate the new enzyme. Another factor, perhaps the rate of incorporation into the membrane or the addition of the flavin prosthetic group8, must determine the rate at which catalytic activity develops.

We conclude that the stepwise synthesis of succinate dehydrogenase is initiated by a brief period of transcription which begins perceptibly before the 2 h period of translation; however, enzyme activity accumulates for a total of 9 h because there is a further, rate-limiting, step in the formation of active; membrane-bound enzyme.

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#### Feedback control of vitamin D metabolism by a nuclear action of 1,25-dihydroxycholecalciferol on thidney

1,25-DIHYDROXYCHOLECALCIFEROL (1,25 DHCC the most active metabolite of vitamin D<sub>3</sub><sup>1-4</sup>, and its productiothe kidney in vivo varies with the calcium and vitamin D ent of the diet<sup>5</sup>. In spite of several theories, the mechani by which dietary changes cause alterations in 1,25 DH6roduction are not completely understood. Hormonal<sup>6-8</sup>1 ionic<sup>9-11</sup> messengers have been suggested, and both maive a role.

In a previous report<sup>10</sup>, we described inhibition he conversion of 25-hydroxycholecalciferol (25 HCC) t25 DHCC in vitro after incubation of isolated chick kidnabules with 1,25 DHCC. We show here that this inhibition of due to a direct competitive effect of 1,25 DHCC, but that probably due to an action on the nucleus. Because of a depence of this effect on the calcium ion concentration, and by agy with the mode of action of 1,25 DHCC in the intestine, it ioposed that 1,25 DHCC induces the formation of proteins alved in calcium movement into and across the cell. The km sensitivity of the 25 HCC-1-hydroxylase enzyme to calcium uggests that this enhanced calcium entry into the cell may eain the inhibitory effect of 1,25 DHCC on the 25 HC(hydroxylase enzyme. The proposed mechanism would exm the interdependence of 25 HCC-1-hydroxylase activity, the calcium and vitamin D nutritional state observed in vivo

Table 1 Effect of preincubation with 1,25 DHCC (5 nmol 1<sup>-1</sup> in (a) and 25 nml 1<sup>-1</sup> in (b)) for (a) different time intals, and at (b) different calcium concentrations on the conversion of6-(27)-methyl-3H)-25 DHCC to 1,25 DHCC by isolated renal tubu from vitamin D-deficient chicks

	D done.c	111 01110110	
Experimental situation	1,25 DHCC	Recovered radi activity (%) as DHCC (Mea ± s.e.m.)	
(a)			
10 min		$30.6 \pm 0.8$	
	+	32.0 + 1.2	n.s.*
30 min		$32.8 \pm 1.0$	
20	+	$35.4 \pm 1.0$	n.s.
150 min		$41.8 \pm 5.1$	
	+	$21.3 \pm 3.3$	P < 0.02
(b)	•		
0.6 mmol I <sup>-1</sup> Ca <sup>2+</sup>		$11.8 \pm 0.3$	
0,0 11111011	+	11.6 + 1.2	n.s.
1.2 mmol 1 <sup>-1</sup> Ca <sup>2+</sup>	<u> </u>	$14.1 \pm 1.0$	
112 11111011	+-	$8.6 \pm 1.9$	P < 0.05
3.6 mmol l <sup>-1</sup> Ca <sup>2+</sup>		$14.5 \pm 0.5$	
510 11111011 04	+	$11.4\pm0.7$	P < 0.01
	•		

The tubules were preincubated for 6 h in mea with different calcium concentrations. The tritiated 25 HCC waladded after the preincubation period and the conversion to 1,25DHCC assessed 15 min later. 1,25 DHCC was added in 5 µl ethanl, and an equal volume of ethanol was added to tubes not containing 1,25 DHCC. Incubation, extraction, separation and counting is the radioactive metabolites were performed as described previous, 10 except that an incubation volume of 1.5 ml was used. It is imporant to note that the tubules remained biochemically viable for at least 6 h, as assessed by ATP levels after different periods of incubation (15 min, 1.91 µg mg<sup>-1</sup> protein; 1 h 2.09 µg mg<sup>-1</sup>; 3 h, 1.44 µg mg<sup>-1</sup> 6 h, 1.68 µg mg<sup>-1</sup>) and <sup>14</sup>CO<sub>2</sub> production from [U-<sup>14</sup>C] glucose whichwas linear for 8 h. Statistical comparisons were made by the unpaired Student's t test.

\* Not significent \* Not significant.

Competinhibition was eliminated in several ways. There is a lag between exposure of the tubules to 1,25 DHCC arhibition of 25 HCC-1-hydroxylase activity (Table 1a) his and other experiments it was found that a minimum omin preincubation in 1,25 DHCC was necessary before inhon of 25 HCC-1-hydroxylase activity was apparent. Time lag was not due to delay in entry of 1,25 DHCC inte cell, as in broken cell preparations (homogenates), 1)HCC in concentrations up to 125 nmol 1-1 (50 ng ml-1 no acute effect on 25 HCC-1-hydroxylase activity (outublished observations). Finally, as seen in Fig. 1, the possibilit the observed inhibition being competitive in type was cetely eliminated by showing that the effect of 25 nmol 1-1 25 DHCC on the rate of the reaction was not reduced by asing the substrate concentration from 12.5 to 150 nmol 1

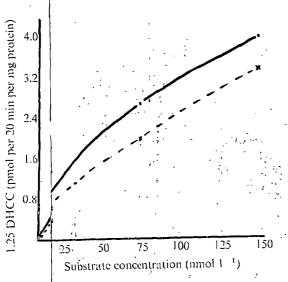


Fig. 1 Relianship between substrate concentration and the rate of the hversion of 25 HCC to 1,25 DHCC in isolated chick renal jules. —, after 6 h preincubation in buffer and —, after h preincubation in buffer containing 25 nmoll<sup>-1</sup> of 1,25 DHC. In the presence of 1,25 DHCC, the rate of the conversion is reduced at each substrate concentration. In This experient, biosynthetically prepared 1,25 DHCC was used (prepar and donated by Dr E. B. Mawer, Department of Medicine, Uversity of Manchester), and its purity was checked by high spe liquid chromatography (E. W. Matthews et al. unpublished).

The possibly that the time lag in onset of the inhibitory effect of 1,25 HCC was due to a nuclear interaction with the renal cell respisible for the 25 HCC-1-hydroxylase activity was investigated studying the effect of actinomycin D. This antibiotic is relatively specific inhibitor of DNA-directed RNA transcrition<sup>12,13</sup>. The concentration used (5.4 µmol l<sup>-1</sup>) inhibited 97 of 5-3H-uridine incorporation into acidprecipitable INA after 30 min exposure of the tubule cells. Exposure to a inomycin D for 6 h did not increase 1,25 DHCC production, ht it did abolish the inhibitory effect of exposure of te tubules to 1,25 DHCC for 5.5 h (Table 2). Although exprience has taught caution in the interpretation of the results of periments using inhibitors of RNA or protein synthesis, the acts that basal 25 HCC-1-hydroxylase activity was not reduced, and 14CO2 production from [U-14C] glucose is not affected, aggest that actinomycin D was not acting as a non-specific toin. The results support the concept that the inhibitory effect of 1,25 DHCC on its own formation might be mediated by a luclear effect of this steroid hormone in initiating new RNA transcription.

A nuclear effect of 1,25 DHCC could not lead to inhibition of 25 HCC-1-hydoxylase activity by preventing the formation of the messenger RNA for the 25 HCC-1-hydroxylase enzyme, because virtualy complete interruption of all new RNA formation by actiomycin D did not depress the enzyme activity.

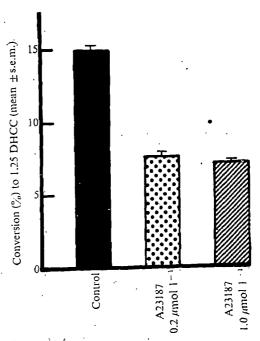


Fig. 2 Effect of the calcium ionophore A23187 on the conversion of 25 HCC to 1,25 DHCC. The preparation was preincubated for 15 min before the addition of tritiated 25 HCC and a further 15 min incubation. Cellular respiration, as assessed by <sup>14</sup>CO<sub>2</sub> production from [U-<sup>14</sup>C]glucose, was unaffected by the ionophore.

The most likely explanation is that, as in the intestine<sup>14,15</sup>, 1,25 DHCC increased the synthesis of proteins concerned with calcium transport<sup>16</sup>. This could result in inhibition of the 1-hydroxylase enzyme, which is known to be sensitive to calcium ion *in vitro*<sup>8,9</sup>. The demonstration of a vitamin D-dependent calcium-binding protein in the kidney is consistent with this view<sup>17</sup>.

The possibility that calcium ion influx could account for inhibition of 25 HCC-1-hydroxylase activity in intact cells was tested by studying the effect of the ionophore A23187<sup>18</sup>. This agent forms lipophilic complexes with calcium (and magnesium) ions, allowing their rapid entry into cells. This agent markedly inhibited 25 HCC-1-hydroxylase activity (Fig. 2), presumably by promoting calcium ion entry. Finally, the experiment shown in Table 1 (b) demonstrated that the inhibitory action of 1,25 DHCC was abolished by lowering the extracellular calcium ion concentration of the medium.

Figure 3 summarises the scheme proposed for regulation of 25 HCC-1-hydroxylase activity at the cellular level. Reabsorption of the calcium from the tubular lumen is enhanced by 1,25 DHCC, presumably by its effect on the synthesis of proteins involved in calcium transport. However, if the calcium concentration in the tubular lumen at the site critical for the hydroxylation of 25 HCC is low, 1,25 DHCC production is unaffected. But if the renal tubular calcium rises, sufficient calcium may be reabsorbed to suppress 1,25 DHCC production. This effect on

Table 2 Prevention by actinomycin D of the effect of 1,25 DHCC on conversion of 25 HCC to 1,25 DHCC in chick kidney tubules

CONVENSION OF 25 THE CONT		Significance
Agent	Recovered radioactivity (%)	Significance
:	as 1,25 DHCC	
•	(Mean $\pm$ s.e.m.)	
Control 1,25 DHCC (25 nmol l <sup>-1</sup> )	$18.5 \pm 1.3 \\ 12.0 \pm 1.4$	P < 0.01
Actinomycin D (5.4 µmol 1 <sup>-1</sup> ) Actinomycin D (5.4 µmol 1 <sup>-1</sup> )+	$17.9 \pm 0.6$ $17.1 \pm 1.0$	n.s.* n.s.
1,25 DHCC (25 nmol 1 <sup>-1</sup> )		

Actinomycin D was added 30 min before 1,25 DHCC; there was a further 5.5 h preincubation prior to the addition of tritiated 25 HCC. In addition actinomycin D (5.4  $\mu$ mol l<sup>-1</sup>) does not impair  $^{13}$ CO<sub>2</sub> production from [U- $^{14}$ C] glucose by the tubules at 3 h or 6 h incubation (unpublished results).

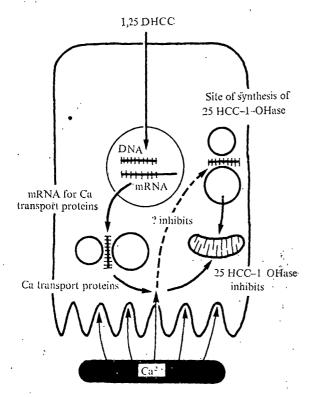


Fig. 3 Schematic representation of a renal tubule cell showing proposed action of 1,25 DHCC on reabsorption of calcium and 25 HCC-1-hydroxylase activity. Other actions of 1,25 DHCC on the tubule cell, such as induction of another hydroxylase enzyme, 25 HCC-24-hydroxylase, are also possible, but are not shown as our in vitro experiments gave no direct evidence for them. Similarly an effect at the translational level may exist and is indicated by

enzyme activity could be followed by a delayed action on enzyme synthesis. In the absence of 1,25 DHCC, it would be predicted that only very high plasma and urinary calcium concentrations could inhibit 25 HCC-1-hydroxylase activity. This is consistent with in vivo observations<sup>5</sup>.

Thus it is proposed that 1,25 DHCC has a dual role in the kidney. It enhances calcium conservation by increased synthesis of calcium transport proteins and, perhaps more importantly, it allows the renal cell responsible for the production of 1,25 DHCC to respond appropriately to normal or high urinary calcium concentrations by reducing the rate of formation of 1,25 DHCC.

These experiments, and the hypothesis they have led to, do not imply that other control mechanisms do not exist. It has become apparent that the regulation of 1,25 DHCC production has multifactorial control, as have most other endocrine systems, and hormonal factors may also be important. The results do however, suggest a teleological explanation for the siting of the 25 HCC-1-hydroxylase enzyme system in the kidney tubule, where it is able to sense and respond to fluctuations in calcium concentration in the main outflow site from the extracellular compartment, and to circulating 1,25 DHCC concentrations.

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#### Erroneous base-pairing induced by chemical carcinogen during DNA sthesis

The stability of binding of chemical carcinoge to DNA1-5 in vivo suggests a molecular mechanism for turn initiation. This interaction may be mutagenic in that it ay affect the accuracy with which the modified DNA is cord by cellular DNA polymerases. We now report that the mification of a synthetic polynucleotide template with  $\beta$ -propiolone increases

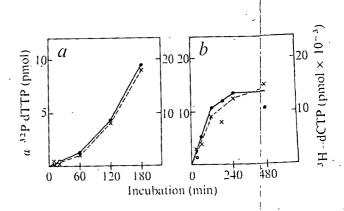


Fig. 1 Time dependency of complemental and non-complementary nucleotide incorporation. a, AMV DNA polymerase, 1 μg of 85 μM modified poly(dA) ligo(dT) was incubated at 37°C in a mixture containing equal quantities of complementary and non-complementary nucleotide concentrations. b, Sea urchin nuclear DNA poymerase, 1 µg of 43 µM modified template was usd.

The assays for simultaneous incorporation of omplementary and non-complementary nucleotides are deailed in the legends to Tables 1 and 3. •, Complementary nucleotide incorporation; x, non-complementary nucleotid incorporation.

Modification of poly(dA) oligo(dT) template	Complementary nucleotide (dTTP) incorporation (pmol)	Non-complementary nucleotide (dCTP) incorporation (pmol)	Error rate
No BPL	6.95	0.012	1/579
9 µM BPL	5.12	0.012	1/426
43 µM BPL	4.96	0.023	1/215
85 µM BPL	5.50	0.027	1/203

The incorion of complementary (dTTP) and non-complementary (dCTP) nucleotides by AMV DNA polymerase was measured at 37° C for 60 min. Taction mixtures (total volume 0.05 ml) contained 50 mM Tris-HCl (pH 8.0), 5 mM MgCl<sub>2</sub>, 20 mM KCl, 5 mM dithiothreitol, 2 µg bovine albumin, 26 µM α-32P-dTTP (20 d.p.m. pmol<sup>-1</sup>), 30 µM 3H-dCTP (55,000 d.p.m. pmol<sup>-1</sup>), 0.1 mg of AMV DNA polymerase, and I ug of ned or unmodified poly(dA) oligo(dT). Samples were washed as previously described14. The values given represent the average of triplicate minations. The error rates were determined by simultaneous incorporation of dTTP and dCTP and varied by less than 10%.

the incorpon of non-complementary base-paired nucleotides into I with the DNA polymerase from either avian myeloblastovirus (AMV) or sea urchin embryos. Homogeneous AIDNA polymerase and partially purified sea

Table 2 Rein requirements for non-complementary nucleotide corporation with modified templates

	•	pmol Inco	orporation
	Reaction	Correct	Incorrect
Enzyn	mixtures	(dTTP)	(dCTP)
A MCV TONTA	Complete	22.5	0.048
AMV DNA rmerase	Minus Mg <sup>2+</sup>	< 1.0	< 0.001
	Minus dTTP	< 1.0	< 0.001
ł	Minus enzyme	< 1.0	< 0.001
G 11	Minus template	< 1.0	< 0.001
Sea urchin nuar	Complete	< 20.6	< 0.0175
DNA polymee	Minus Mg <sup>2+</sup>	< 1.0	< 0.002
	Minus dTTP	< 1.0	< 0.002
1	Minus enzyme	< 1.0	< 0.002
1	Minus template	< 1.0	< 0.002

The compil reaction mixtures and incubation conditions are given in Tabl 1 and 3. The absolute error rates of AMV DNA polymerase area urchin DNA polymerase should not be compared because partily purified sea urchin enzyme was used in these studies.

urchin nucleaDNA polymerase, which are devoid of nuclease activity<sup>6,7</sup> we used. β-Propiolactone is a potent mutagen<sup>8,9</sup> and carcinon, initiating tumours in mice10, rats11 and hamsters12. Is an alkylating agent that reacts with guanine and adenine icleotides in DNA13.

β-Propiolapne (0-85 μM final concentration) was incubated with 200  $\mu$ g f poly(dA) $\frac{1}{2000}$  oligo(dT)<sub>12-18</sub>, mass ratio 1:1, for 20h at 3 C in the dark. Unbound  $\beta$ -propiolactone was removed by dlysis for 24h at room temperature. Studies with 3H-β-propiolitone (Searle, Amersham) demonstrate that at a final concentration of 43 µM one molecule was bound per 180 nucleotide residues in the template. All radioactivity after dialysis was acid-precipitable. As a control, poly(dA)2000 oligo(dT)<sub>12-18</sub> was incubated without β-propiolactone; and the modified and unmodified poly(dA)<sub>2000</sub>·oligo(dT)<sub>12-18</sub> were dialysed together. Size distributions of the unmodified and carcinogen-modified templates were similar as determined by zonal sedimentation in neutral sucrose gradients.

The accuracy of DNA synthesis with AMV DNA polymerase and modified poly(dA)<sub>2000</sub>·oligo(dT)<sub>12-18</sub> was determined (Table 1). Synthesis was measured by the simultaneous incorporation of the complementary ( $\alpha$ -32P-dTTP) and non-complementary (3H-dCTP) nucleotides. As previously reported, AMV DNA polymerase incorporates an unusually large number of non-complementary nucleotides14. With  $poly(dA)_{2000} \cdot oligo(dT)_{12-18}$  as a template, AMV DNA polymerase incorporates one molecule of non-complementary nucleotide (dCTP) for approximately every 600 molecules of complementary nucleotide polymerised. We found that the number of non-complementary nucleotides incorporated increased progressively as template modification was increased. This error rate of 1/579 increased to 1/203 as a result of treatment with β-propiolactone. Modification with increasing amounts of  $\beta$ -propiolactone did not significantly decrease the incorporation of complementary nucleotides. Although the absolute error rates varied as much as twofold in experiments with different preparations of enzyme, a relative increase in error rates with carcinogen treatment similar to that shown in Table 1 was observed in all experiments.

The reaction requirements for the incorporation of noncomplementary nucleotides with carcinogen-modified templates are shown in Table 2. The incorporation of non-complementary nucleotides was totally dependent on Mg2+, correct nucleotide (dTTP), enzyme and template. Elimination of any of these components eliminated detectable incorporation of noncomplementary nucleotide. These requirements are similar to those reported for unmodified templates14 and suggest that polymerisation is required for the incorporation

Table 3 Response of sea urchin nuclear DNA polymerase to carcinogen-modified poly(dA)·oligo(dT)						
Modification of poly(dA) oligo(dT) template	Complementary nucleotide (dTTP) incorporation (pmol)	Non-complementary nucleotide (dCTP) incorporation (pmol)	Error rate			
No BPL 9 µM BPL 43 µM BPL 85 µM BPL	37.36 56.31 33.68 17.89	0.0097 0.023 0.016 0.011	1/3852 1/2448 1/2105 1/1626			

Complementary (dTTP) and non-complementary (dCTP) nucleotide incorporation by sea urchin nuclear DNA polymerase was measured at 28° C for 8 h The reaction mixture (0.05 ml total volume) contained 40 mM Tris-HCl (pH 8.0), 4 mM MgCl<sub>2</sub>, 6 mM dithiothreitol, 50 mM  $\alpha$ -32P-dTTP (38d.p.m. pmol<sup>-1</sup>), 50 mM <sup>3</sup>H-dCTP (2.74×10<sup>3</sup> d.p.m. pmol<sup>-1</sup>), 10% glycerol, 3 µg sea urchin nuclear DNA polymerase, and 1.5 µg of modified or unmodified poly(dA) · oligo(dT). Samples were washed according to the procedure of Battula and Loeb<sup>14</sup>. The enzyme used in this experiment corresponds to Fraction V as previously reported<sup>15</sup>.

non-complementary nucleotides. The time dependency of non-complementary nucleotide incorporation was also investigated (Fig. 1a). There was coordinate incorporation of non-complementary and complementary nucleotides, suggesting that these errors are evenly distributed during synthesis. The product of the reaction was digested by snake venom phosphodiesterase. Chromatographic analysis demonstrated that the 3H and 32P radioactivity in the product was recovered as <sup>3</sup>H-cCMP and <sup>32</sup>P-dTMP. This indicates that the <sup>3</sup>H count in the product is indeed dCMP and that the non-complementary nucleotide is present in phosphodiester linkage.

To test whether this increase in errors in DNA synthesis was unique to AMV DNA polymerase, we also used sea urchin nuclear DNA polymerase. As Table 3 shows, the error rate with unmodified poly(dA) oligo(dT)<sub>12-18</sub> was 1/3852 and could be increased to 1/1626 with more extensive template modification. When the template was treated with the carcinogen there was an initial stimulation in synthesis followed by a decrease in synthesis as template modification increased. The incorporation of the non-complementary nucleotide was dependent on Mg2+, correct nucleotide, enzyme and template (Table 3). The time dependency of the reaction (Fig. 1b) shows that continued synthesis is required for incorporation.

These results demonstrate a possible relationship between a chemical carcinogen and errors in DNA replication. The accuracy with which a polynucleotide template is copied by AMV DNA polymerase and by sea urchin DNA polymerase is decreased as a result of modification of the template by the carcinogen. Even though the stoichiometry of bound β-propiolactone approximates the increased number of errors observed with AMV DNA polymerase, we have no direct evidence that the alkylated nucleotides are in juxtaposition to the observed errors in the newly synthesised DNA. Thus, a chemical carcinogen may potentiate the mutagenic effects of the AMV DNA polymerase and may directly induce mutations during DNA replication in normal cells.

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#### Early simultaneous appearance of antigen binding cells in the foetal she

THE two classes of theory on the generation of diy in the immune system differ as to when the genetic capfor producing a full repertoire of antibody clonotypes is ved. The germline theory proposes that diversification occainly on an evolutionary timescale at the species level, withimmune capacity of an individual predetermined at tistant of fertilisation; the various somatic theories2-8 pro a much briefer period of diversification from a minimal cement of V genes, occurring during the ontogeny of the idual or even later in life. We have attempted to study the course of generation of diversity by following the appearancymphoid cell receptors binding any of several antigens in fisheep.

If one assumes that cell-surface receptors for en reflect accurately the antibody specificities produced by ells, then, according to the germ-line theory, one predictioned be that all receptor specificities should arise simultaneous soon as the immune system had developed to the powhere the receptors could be displayed on the surfaces of hoid cells. According to somatic theories, the appearand various receptor specificities might follow several diffe patterns. (a) If diversification occurred randomly, then in ane animal there should be a random accumulation of receptecificities following no set time course, except for the earlyression of certain high probability 'hot-spot' mutations or gic changes few steps removed from the original, small gerne set of Vgenes. (b) If diversification were antigen driven, the number of receptor specificities and the frequency of aen-binding cells (ABC) for related antigens should increasith antigen exposure. (c) Another possibility would be primitive antigen receptors, which bind weakly to a la variety of antigens, would be replaced, as genetic diversifica occurred, by receptors which would discriminate much te precisely among different epitopes.

Silverstein has reported that the maturaticof immune competence in the foetal sheep, as measured bhe animal's ability to make antibody, does not occur at thene stage of development for all antigens, but follows a partier sequence: anti- $\Phi X174$  antibody can be made at day 35 gestation, anti-ferritin at day 65, anti-ovalbumin at day i, and antibodies to Salmonella and BCG only several we after birth (where full term is about 150 d). A hierarchy of ponsiveness to nine bacteriophage, hapten and protein anths has also been described in the opossum<sup>10</sup>. The stage of elopment is quite different, however, at which the opossum st responds to two antigens also used in the sheep studic The events leading up to antibody synthesis include the interion of T and B lymphocytes and macrophages; therefore, a la of response could be due to a deficiency of receptor-geneing cells or

essential accessory cells9.

We chose to look for cells capable of specially binding antigen, among which population the precursorof antibodyforming cells and T helper cells would be expecte to be found, to see how early in ontogeny such cells were resent in the foetal sheep and whether their appearance aralleled the appearance of response capacity measured by amody production. It has already been demonstrated that this heep has a proportion of Ig-bearing lymphocytes in its peripiral lymphoid organs similar to that in the mouse11, and we lve confirmed this with our fluorescent anti-sheep Ig reagent. Ince the sheep has a placental barrier to maternal immunglobulin9, the appearance of foetal antigen-binding cells ABC) should reflect the genetic capacity of the foetus to make specific antigen recognition units. Single cell suspensionswere prepared from the lymphoid organs of foetuses from tim-bred sheep at 58, 87, and 140 d gestation, and from the spleer of two of the ewes. The cells were fixed with glutaraldehyde to preserve their receptors<sup>12-14</sup>, incubated with 500 µg ml<sup>-1</sup> fluorchrome-conjugated antigen, and observed for ABC with a leitz Ortholux

fluorescericroscope equipped with Ploem-type vertical illuminatiFor a more detailed presentation of the methods, see DeLual. (submitted for publication)).

Table is the % ABC observed in the spleens of the foetal andt (pregnant) sheep. ABC were observed for all four antigs early as day 58 of gestation, including ovalbumin forh no antibody formation can be detected until 125 d ges. Splenic ABC were always Ig-bearing cells; this was examined in a 140-d foetal sheep and its dramatic increase in ABC is seen as the foetus des. ABC frequencies are higher in the adult spleen (eally in the 140-d pregnant ewe), perhaps as a respf exposure to cross-reacting antigens, a change in cell types constituting the spleen, or changes associated pregnancy as observed in the mouse<sup>16</sup>. Binding of HSF, and GZ were shown to be non-competitive by inhibition riments in which a 10-fold excess of HSF or a 40-fold exof OA prevented the binding of rhodamine-HSF or fluoresc)A, respectively, but not the binding of fluorescein-GZ. Fescein-HSF and rhodamine-HSF gave the same frequency BC with adult spleen cells.

ABC for and HSF were observed in the thymus, marrow and liver all as the spleen of the 58-d foetal sheep (Table 2). Two diffens from ABC frequencies in foetal mice16 are readily appt. First, the ABC frequency in the foetal sheep liver is verw, and many ABC are present in the marrow; even late instation, foetal mice have 0.1-0.5% ABC in the liver and vally no cells in the marrow. Foetal sheep liver contains my large hepatocytes at 58 d, contrasted to foetal

Table 1	Sp	c antigen-	binding cells in	foetal and	adult sheep		
4 ~~			% A	% ABC*			
Age	ĺ	GZ†	HSF‡	KLH‡	OA§		
58 d		0.08	0.14	0.13	0.04		
87 d	- 1	80.0	0.06	0.03	0.07		
140 d	- }	0.10	0.10	0.11	0.20		
Adult ∥	- 1	0.54	1.0	0.35	0.25		
Adult ¶	Į	2.9	2.4	2.8	0.8		

\* At least ABC or 10,000 total cells were counted for most samples.

† E. coli β-actosidase, extracted from strain A-3245 and purified by affinity chmotography. ‡ Horse spin ferritin and keyhole limpet haemocyanin. A grade

(Sigma), § Ovalbum 5× crystallised (Pentex).

li 58 d pregnt. ¶ 140 d preant.

Cell prepanions, antigen incubation, and washes were done in buffered balaed salt solution, pH 7.2, containing 0.25% gelatin. Cells were fix with 0.2% glutaraldehyde in 0.1 M phosphate buffer pH 7.0 for min; antigen incubation was for 30-60 min. All procedures we carried out in the cold. The sheep were obtained

from Weern Scientific Breeders, Inc., Davis, California.

mouse liver hich contains mostly haematopoetic stem cells. The second evelopmental difference is in the thymus. Mice have high AC frequencies (of the order of 1%) when the thymus can rst be studied (14 d = 70% term), declining to 0.1% by late estation and birth. At 58 d (40% term), 20 d after thymocytes 1st are observed in the thymic rudiment17, the thymic ABC requency in the foetal sheep is quite low. It may be that a decine in ABC frequency similar to that observed in mouse and human foetal thymus 16,18,19 has already occurred by 58 d gestation in the sheep.

The data presented above suggest that the inability of the foetal sheep to make an antibody response to all antigens early in development is probably a result of a deficiency in antigen handling or cell interactions, a requirement which may be more stringent for some responses than others, rather than to a lack

of B or T lymphocytes which can recognise antigen. No evidence exists for a sequential appearance of ABC for different antigens; although foetal sheep first respond to HSF at day 65 of gestation and to OA at day 125, ABC for both antigens are present on day 58. In these early ABC, the inhibition of specific antigen binding by nonlabelled homologous antigen and its absence of effect on the binding of unrelated antigens is not compatible with the concept of a primitive receptor, capable of binding many different epitopes.

Table 2 ABC in several lymphoid organs of 58 d foetal sheep %ABC\* GΖ HSF Organ 0.09 0.05 Thymus 0.60 1.0 Marrow 0.08 0.14 Spleen 0.009 < 0.02

\* At least 10 ABC or 10,000 total cells were counted for most samples.

The early appearance of ABC for a variety of antigens in mice and man16,18,19, as well as the sheep, argues against a requirement for a long period of somatic diversification to produce most of the receptor clonotypes the individual possesses. It is possible that the use of three large antigens might exaggerate the proportion of the receptor universe present at the earliest time. But our studies reported here with ovalbumin, and experiments with early foetal binding of horse radish peroxidase16 and flagellin18 seem to vitiate this objection.

We conclude that the ability to bind antigen is one of the earliest events in immune development, and that the genetic potential to respond to a wide variety of epitopes is predominantly germline in nature. The apparently orderly development of the capacity to respond to different antigens by antibody production must reflect a biological clock which modulates T cell-B cell-macrophage interactions and not the regulated acquisition of particular V-gene regions.

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#### Presence of J chain in human immunocytes containing various immunoglobulin classes

THE polypeptide J chain is common to dimeric IgA and polymeric IgM<sup>1-3</sup>. Immunocytes producing such immunoglobulins have been shown to synthesise J chain<sup>4-8</sup>; its incorporation has therefore been assumed to be an essential step in the intracellular joining of the monomer (H2L2) subunits. But the finding of a monoclonal polymeric IgM lacking J chain<sup>7,8</sup>, and reassociation of IgM subunits depleted of J chain<sup>8</sup>, have led to questioning of its role as an initiator of polymerisation. Experiments demonstrating J chain in four murine IgG myeloma cell lines10 prompted me to examine its cellular localisation in human biopsy material. Cytoplasmic J chain was detected in IgD, IgM, IgG, and IgA immunocytes by direct immunofluorescence. This localisation was compared with the cytoplasmic ability to bind "secretory component" (SC) in vitro in a SC-affinity test that has been postulated to distinguish between polymer and monomer producing cells<sup>11</sup>.

Human J chain was purified from dimeric IgA by reduction with 10-20 mM dithiothreitol, gel filtration and immunoadsorption with an insolubilised antiserum to human serum12. Four rabbits each received subcutaneous injections of 0.4 mg J chain coupled to bovine serum albumin (BSA)13 and emulsified in Freund's complete adjuvant. The same dose was given after 4 weeks; and 1 month thereafter 1 mg uncoupled J chain was injected. All rabbits had an appreciable antibody titre 14 d subsequently. After adsorption with BSA and insolubilised serum from a patient with hypo-y-globulinaemia, the antisera were shown to be monospecific to J chain, giving an identity reaction with reference antiserum provided by Dr J. Mestecky. IgG from the most potent antiserum was labelled with fluorescein isothiocyanate, and a fraction of this 'green' conjugate with an absorbance ratio of 2.2 was used at 0.6 mg and 1/10 precipitating units per ml (ref. 14). Human tissue specimens were extracted for 48 h at 4° C in isotonic phosphate-buffered saline, fixed in 95% alcohol, and embedded in paraffin<sup>15</sup>. Serial sections were incubated with a combination of the 'green' anti-J-chain and a 'red' (rhodamine) conjugate monospecific to human IgG, IgA, IgM or IgD14.15. Other sections were first incubated with SC, and thereafter with a combination of 'red' anti-SC and 'green' anti-Ig to identify cells with cytoplasmic affinity for SC11,16. The sections were examined in a Leitz Ortholux microscope equipped with a Ploem-type vertical illuminator and interference filters for narrow-band excitation and selective filtration of green or red fluorescence<sup>15</sup>.

The SC affinity test distinguished between three types of tissue with regard to IgA cells: (1) immunocytes associated with glands in colon and nasal mucosa were generally positive11;

(2) tonsils contained chiefly negative IgA immunorut also positive cells scattered or in groups11; and (3) cells in inflammatory infiltrates did not generally bind Se most 'pure' cell population of the latter type was in the gingiva which is a part of the oral mucosa complacking glands. This tissue is prone to chronic inflammaticause of contact with the bacterial plaque on the teeth. Inmunocytes associated with glands, also bound SC, whthe few such cells present in the gingiva were equallitive or negative. We interpreted these findings as inng that IgA and IgM immunocytes of glandular areas predimeric or polymeric immunoglobulins containing Jh. This assumption was based on the fact that isolateneric or polymeric IgA and 19S IgM combine spontally with SC in test tube experiments; but apparently othen the immunoglobulins contain J chain<sup>8,16</sup>. Monomocluding 7S IgA, do not combine with SC. By inference rigA cells in the gingiva should produce monomeric IgA.

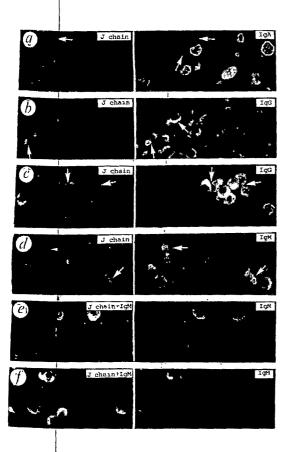
These conclusions were supported by the dimmunohistochemical demonstration of J chain. In the ga only a few (3-8) out of many IgA cells in each section fined this peptide (Fig. 1a), a number comparable with that t-binding cells. J chain could moreover be detected in onlout 50% of the few gingival IgM immunocytes. This casts with glandular areas in the nasal mucosa and colorere most IgM (Fig. 1d) and IgA cells were positive. The irity of the IgA cells was generally weak and varied considers however, possibly reflecting different concentrations of peptide (Fig. 1e). In the tonsils small groups of cellsitaining J chain especially beneath the epithelium, correspond to areas where some SC-binding IgA immunocytes were ent. These results substantiated that IgA cells appearing inflamed gingiva and tonsils are chiefly monomer proers which cannot synthesise J chain.

Many IgG cells characteristically appear in fof chronic inflammation such as in the gingiva<sup>17,18</sup> and aw (<1%) definitely contained J chain (Fig. 1b). In the supcial stroma of nasal mucosa IgG immunocytes accumulate a result of rhinitis<sup>19</sup>; 30-60% of these cells were J chain pucers (Fig. 1c). Also many of the few IgG immunocytes agent to the crypts in normal colon mucosa were J chain-litive. This was also true for IgG cells found along withM cells in some of the lymphoid follicles in the tonsils. But, two out of the four specimens (one pharyngeal and one latine) the number of positive cells was too large to be accited for by IgM, IgG and IgA immunocytes (Fig. 1f). In the two specimens cells containing IgD outnumbered thoswith IgM, especially between the lymphoid foilicles and thurface; and the majority of the IgD immunocytes were ind J chain-

The specificity of the J chain immunofluccence was established as follows: (1) Addition to the conjute of highly purified J chain (1 mg ml-1) blocked the stang reaction completely; (2) addition of comparable amount of F(ab')2 had no effect; (3) the simultaneous demonstration of J chain positive and negative immunocytes of each Ig iss excluded the possibility of cross reactions with parts of the or H chains. Furthermore, none of our conjugates produced filrescing cells when applied in working dilutions to section of a nasal polyp from a patient with hypo-γ-globulinaemia

We have thus for the first time provided eviden that J chain may be produced by immunocytes of any Ig ass with the possible exception of IgE. This accords with weral studies indicating that B cells of a specific clone are gertically multipotent with regard to their H chain product<sup>20</sup>. Mny circulating B cells have both IgM and IgD in their surface mmbranes 21,22; and the simultaneous presence of IgM in the nembrane and IgG in the cytoplasm has also been described3. In general, however, the secretory product of a single mattre B cell is of only one Ig class and it has been postulated tha there may be an intraclonal switch from expression of IgM synthesis to IgG, and perhaps to IgA24. The finding of IgD-conaining plasma

cells adjac lymphoid follicles indicates that IgD may be expressed in such a development. The presence of J chain in most of cells moreover suggests that this polypeptide represents basic gene product of B cells, the synthesis of which is ried only after prolonged clonal proliferation. In lympholicles and adjacent to glandular epithelia, continuous cl expansions from immunocytes that have recently swl their H chain product may occur as a result of frequent ptation of new antigenic material. This is in



Immohistochemical demonstration of J chain in human tissi. Paired staining was performed with a 'green' anti-J-chain njugate combined with a 'red' conjugate specific for IgA, Iglor IgM. Right column: records of selective red emission. Lecolumn: a-d, records of selective green emission; e, f, double posures. a, Field from gingival section with two intense J qin-positive cells (arrows) which are not IgA immunocyte b, Comparable field from section adjacent to a showing a w J chain-positive IgG immunocytes (arrows). c, Area beath the surface epithelium of nasal mucosa containing to intense (left arrow) and three faint (right arrow) J cin-positive IgG immunocytes. d, Field from glandular aa of inflamed colon mucosa (Crohn's disease) containing imerous J chain-positive IgM immunocytes. Left arrow, dicates a negative cell, and right arrow, a positive one. e, Fieldrom glandular area of normal colon mucosa with two IgM ces which are stained brightly for J chain (mixed colour in lefpicture), and numerous more or less faint, green cells which a neighbouring section were shown to be IgA immunocytes, Part of an area adjacent to a tonsillar lymphoid follicle. The were a few J chain-positive IgM cells (two shown), and everal other immunocytes brightly fluorescing for J chain. Staing of neighbouring sections demonstrated that most of tese contained IgD, and some contained IgG.

accordance wih the fact that the largest number of J chainpositive IgG cells were found in these areas.

Studies of nouse and human cell lines have provided contradictory information with regard to the intracellular events in the Ig polymerization process<sup>5,25,26</sup>. The SC-affinity test directly

demonstrates that normal gland-associated human IgA and IgM immunocytes must contain cytoplasmic subunits in an arrangement with J chain comparable to that constituting the SC-binding site of the extracellular immunoglobulins<sup>16</sup>. For IgM3, as well as IgA, this arrangement may be a dimer which is not necessarily covalently stabilised initially. If this dimeric configuration is likewise expressed in the membrane of IgM and IgA cell precursors, these B lymphocytes may by contact with SC-producing epithelial cells or interstitial free SC, receive a stimulus on their mucosal recirculation route, which induces their settlement and local proliferation. In addition the J chain-dependent SC-binding site of extracellular immunoglobulins may mediate the epithelial reception and the selective glandular transmission of dimeric IgA and 19S IgM<sup>15,27</sup>. This peptide therefore seems to be important in the evolution of the mucosal immune system.

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#### Anti-tumour cytotoxic effects mediated by minor and major cell populations of lymph nodes

SEVERAL studies have demonstrated that cell-mediated immunity to animal and human tumours can be measured in vitro by colony inhibition or microcytotoxicity tests in which immune lymphoid cells kill and/or inhibit the growth of tumour cells<sup>1,2</sup>, and more recent studies have attempted to extend these results by measuring the relative contributions of T and B lymphocytes to the anti-tumour effects noted<sup>3,4</sup>. The principle upon which these latter experiments have been based is that selective depletion of one or the other lymphocyte subpopulation, followed by measurement of the capacity of the residual population to produce an effect, should indicate which subpopulation is operative in the system. But, conclusions drawn from such experiments are critically dependent upon the assumption that the remaining lymphocyte subpopulation is not contaminated with other cell types; or, if it is, the contaminating cells are unlikely to be effector cells in the assay used because they are inactive and/or infrequent. Acceptance of this assumption requires careful evaluation; for as we have observed in the present investigation of effector cells operating during the primary immune response to murine Moloney sarcomas, a cell type of low frequency may contribute in a major way to the cytotoxic effects noted in vitro. Furthermore, these cells are present in considerable numbers within regréssing tumours in vivo.

Table 1 Cytostasis mediated by LNC and non-adherent LNC

	MSC		MCA-12	
Effector cells	c.p.m.	%*	c.p.m.	%*
LNC	•	, ,	-	, •
Normal	$7.532 \pm 865$	62†	9.559 + 700	29†
Immune	2.814 + 378		6,733 + 530	
Non-adherent LNC	_,		-, 1	
Normal	$14.043 \pm 1288$	44†	$12,869 \pm 1852$	7(ns)
Immune	$7,748 \pm 605$	. • 1	$11,940 \pm 1189$	. (220)

MSC or MCA-12 tumour cells (800) were cultured for 42 h with 8×10<sup>s</sup> effector cells in Linbro Multidish Disposo trays (0.2 ml per well); trays were washed by immersion, surviving tumour cells were pulsed for 6 h with IUdR (0.2 µCi per well), and trays were washed again. For effects of varying tumour cell and effector cell number in the assay see ref. 6.

LNC were used as such or were passed through divinylbenzene/polystyrene bead columns (37° C) to remove adherent LNC. Non-adherent LNC preparations contain <0.2% phagocytic macrophages and >99% lymphocytes of which 20-25% are Ig and 65are θ-bearing cells.

5% are 6-bearing cens. CPM IUdR (mean±s.d.) remaining adherent at the conclusion of

the cytotoxicity test.

\*Percent cytostasis (or cytotoxicity)= (c.p.m. in cultures with normal effector cells)-(c.p.m. in cultures with immune effector cells)

(c.p.m. in cultures with normal effector cells)

Percentages evaluated by Student's t test: ns=not significant; †P < 0.001.

Moloney sarcomas were induced in 4-week-old female BALB/c mice; and 12 to 18 d later, when tumours were regressing, lymph node cell (LNC) suspensions were prepared and their effects on syngeneic tumour cells were determined as previously described<sup>5-7</sup>. Cell-mediated killing of tumour cells (cytotoxicity test) was measured by labelling o lines of tumour cells with 125I-iododeoxyuridine (IU, DNA isotope, and culturing them with effector cells fh. Cellmediated inhibition of tumour cell growth (cyis test) was assessed similarly except that unlabelled ur cells were cultured with effector cells for 42 h and turviving tumour cells were pulsed with IUdR for 6 h. In assays, the number of surviving adherent tumour cells ctes with the amount of radioactivity remaining adherent; culture surface<sup>8</sup>. We have recently made an extensive as of the variables influencing the results obtained in this system, and the work reported here is based on these find

Whole LNC obtained at the time of tumour reon killed both Moloney sarcoma cells (MSC) and unre tumour cells such as polyoma virus-transformed 3 broblasts (PY)7 and methylcholanthrene-induced sarcomal (MCA-12)6. A representative cytostasis test in which MSMCA-12 were cultured with LNC demonstrates that both kif tumour cells were killed by LNC from mice whose urs were regressing (Table 1). PY and MCA-12 tumour are unrelated antigenically to MSC by the followinteria: (1) serum from mice with regressing Moloney sarce binds to MSC but not to PY and MCA-12 as detected 25I-rabbit anti-mouse immunoglobulin (Seeger, unpublis and (2) neither PY nor MCA-12 has C-type particles orip-specific

Table 2 Treatment of LNC with silica and of non-adit LNC with

٠,	50	anti-0 serum
Experi- ment	- Effector cells	c.p.m.* Treatment: Normal Imm %
1	LNC	None 3,933±254 2,5625 34† Silica 3,672±682 3,1430 14(ns)
2 .	Non- adherent LNC	Normal 6,808±923 4,4427 341 AKR+C'
3	adherent	Anti-0+C 5,028±396 4,42,63 11(ns) Normal 1,059± 85 67,91 35‡ AKR+C
•	) of	Anti- $\theta$ +C' 769± 96 78185 +1 (ns)

Experiment (1): cytostasis test as in Table 1 exc that  $4 \times 10^5$ LNC were cultured without or with silica (40 µg | 1 Min-U-Sil 5 µm diameter (Whitaker-Clark-Daniels)) for 24 h be unlabelled MSC were added. Cultures were then continued 42 h when surviving MSC were avaled with 1112

surviving MSC were pulsed with IUdR.

Experiment (2): cytostasis test performed as in Τε 1. NALNC (5×10°) were treated with anti-θ C3H serum (raisen AKR mice by repeated injections of C3H thymocytes and used a dilution of 1/10 which was on a plateau of killing against BAL/LNC; specificity of antiserum for the  $\theta$  alloantigen demonstrd by showing that absorption with BALB/c brain removed all cyoxic activity) or normal AKR serum (30 min, room temperatur Both groups then were treated with guinea pig complement (1 dilution, 30 min, 37° C). Dead cells were removed by incubon in 0.25% trypsin in Tris buffer (5 min, 37° C) followed by filtron through a chart filter (belief of the magnitude). short filter (boiled cotton wool).

Experiment (3): cytotoxicity test: 2,000 IUdR-labed MSC were cultured with 8×10<sup>5</sup> effector cells for 48 h. Effectcells prepared as in Experiment 2.

Percentages evaluated by Student's t test; ns t significant; < 0.01; ‡P < 0.001.

\*MSC was the target cell in these experiments.

antigen which MSC does have. Therefore, the bserved cytotoxicity is likely to be truly nonspecific and nodue to crossreacting antigens.

By contrast, non-adherent LNC (NALNC) btained from mice whose tumours were regressing killed ISC but not antigenically unrelated MCA-12 (Table 1). lote that the magnitude of killing mediated by NALNC in the and previous experiments6 is less than that mediated by an eual number of whole LNC. Specific killing by NALNC agree with our previously reported observations6,7 and with serol-gically defined specificities.

We sus that the dominant effector cells in whole LNC suspensiore macrophages, even though they account for only 2.5% effector cells, because: (1) activated macrophages kispecifically8,8; (2) nonspecific killing mediated by LNC isbrogated when T lymphocytes are removed by treatment anti-θ C3H serum and complement<sup>7</sup>; (3) macrophae adherent cells, and removal of adherent cells abrogates ecific killing<sup>6,7</sup>; and (4) macrophages are present in greaterbers in immune than in normal control LNC suspension compared with 0.9%) (proportions of macrophagre estimated from cytocentrifuge preparations following ation with polystyrene particles as markers of phagocytoso further test the possibility that macrophages, although ident in number, were the dominant effector cells, wholic were cultured with silica for 24 h to kill macrophag; and then unlabelled tumour cells were added to thaining LNC (Table 2, experiment 1). Because low number NALNC  $(2 \times 10^{5} - 4 \times 10^{5})$  are not detectably cytotoxic is assay system6, we expected that removal of macrophagrom 4×105 LNC by treatment with silica would abre the cytotoxic effect. Indeed, immune LNC treated in a manner did not significantly inhibit the growth of C, whereas untreated immune LNC did so. Control expents indicated that silica was not affecting lymphocyte/ival.

The specialling observed with NALNC is most likely due to lymphocy for such preparations contain > 99 % lymphocytes and Ichan 0.2% macrophages. To determine if this killing was endent on T lymphocytes, NALNC were treated with anti- $\theta$  ( serum and complement (Table 2). In two such experiments e a cytostasis test (experiment 2) and the other a cytotoxicitest (experiment 3), treatment with normal AKR serunnd complement (control treatment) did not affect the diffices between normal and immune NALNC, but treatment wanti-0 C3H serum and complement markedly decreased obmpletely abrogated the differences between them. We inpret this as indicating the dependence of the specific effec NALNC on T lymphocytes. As yet we have no evidence on ether the cytotoxicity mediated by NALNC is due to dit T cell killing or is mediated by other cells whose action pends on T lymphocytes.

A number other recent studies of the immune response to primary Moley sarcomas have been aimed at determining which cell tys mediate cytotoxic reactions in vitro. Lamon

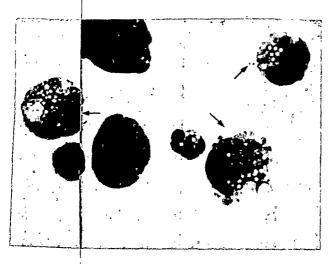


Fig. 1 Cytcentrifuge preparation made from a Moloney sarcoma. The limour was trypsinised to provide a cell suspension which was the incubated with polystyrene beads. The beads then provide markers of phagocytic cells such as macrophages and polymorphoniclear leukocytes. Three macrophages are arrowed; the remainingcells comprise two large cells which are tumour cells and one lymphocyte and one polymorph (containing beads).

Macrophages make up 35% of the cells present.

et al.3, using a microplate system involving visual counting of surviving adherent tumour cells, have reported that immunologically specific inhibition of tumour cell growth was mediated by mixtures of whole LNC and spleen cells during the development and after the regression of Moloney sarcomas. Cell separation experiments suggested that both T and B lymphocytes were responsible for the effects noted. Plata et al.4 have reported similar results in a microplate assay, but in a 51Cr release assay the same group of workers11 and others12 have found that T lymphocytes are the only active population at the time of tumour regression. The reasons why these studies have not detected macrophage mediated effects when using whole LNC or spleen cells is unclear, for when Moloney sarcomas are regressing, LNC and spleen cell suspensions contain 2.5 and 10.1% macrophage respectively?. Even if preparative methods are used to remove adherent and phagocytic macrophages from spleen suspensions, we have found that non-adherent and non-phagocytic precursors soon become mature macrophages capable of cytotoxicity. Possibly the tumour cells used in previous studies3,4 were not susceptible to macrophage-mediated cytotoxic effects. Certainly, the relatively short term 51Cr release assay may be less able to detect macrophage effects than the 48 h test which we use.

Our experiments demonstrate that macrophages, a minor non-T cell subpopulation of lymph nodes, can be potent cytotoxic cells in vitro overshadowing the effects of lymphocytes. This observation suggest caution in interpreting experiments in which effects of different cell types are being assessed if such preparations are contaminated by even small numbers of other cell types. In the host response to primary Moloney sarcomas, cytotoxicity mediated by macrophages is nonspecific, whereas that mediated by lymphocytes is specific and is dependent on T lymphocytes. None the less, the cytotoxic effects of macrophages in vivo may be of considerable importance. Certainly, regressing Moloney tumours contain large numbers of such cells (Fig. 1). We are attempting to clarify the relative importance of macrophages and lymphocytes as effector cells in tumour rejection by studying the cytotoxic potential of cells taken directly from tumours.

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# matters arising

#### Intestinal transport protein

We would like to take issue with the recent letter of Faust and Shearin<sup>1</sup> reporting on a "sugar and amino acid transport protein . . . from hamster jejunum".

The questionable significance of the observed p-glucose binding proteins from the intestine to intestinal Na+-dependent monosaccharide transport has been discussed in detail by Eichholz and Howell<sup>2</sup> and Garcia-Castineiras et al.3. In addition, we would like to point out that it is highly misleading to give a binding protein the adjunct transport when it does not fulfil one of the fundamental requirements for transport, namely high rates of binding and debinding. That the debinding of D-glucose is extremely slow has been previously noted by Eichholz et al.4, and can be deduced from the gel chromatography data of Faust and Shearin<sup>1</sup>. In fact, D-14C-glucose remains tightly bound to a macromolecule during passage through Sephadex G-751. If anything, this tight binding suggests that the "protein" plays a role in storage rather than transport of D-glucose.

That the bulk of the observed pglucose binding may be unrelated to transport is also indicated by one of our observations. We have investigated Dglucose transport in isolated and highly purified vesicles of brush border membrane from rat small intestine5,6. Pre-

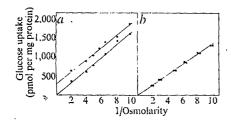


Fig. 1. Effect of medium osmolarity on D-glucose uptake in isolated intestinal brush border membranes. Preparation of membranes (in 100 mM cellobiose, 1 mM HEPES, adjusted to pH 7.5 with Tris, and 0.1 mM MgSO<sub>4</sub>) and the uptake procedure have been previously described<sup>5</sup>. Uptake was measured from a medium with the following final composition: NaSCN (25 mM), Tris-HEPES (1 mM), MgSO, (0.1 mM), D-1-3H-glucose (1 mM) and L-1-14C-glucose (1 mM) and enough cellobiose to give the indicated osmolarity (shown as inverse osmolarity). Time of incubation: p-glucose, 10 min; L-glucose, 20 min; temperature, 25° C. The lines were calculated by regression analysis (correlation coefficient in all 

viously, D-glucose uptake by the isolated membranes had been measured mainly at pH 7.5. When investigating D-glucose uptake as a function of pH we noticed that with lower pH values the equilibrium uptake increased above the pH 7.5 level. This 'extra' D-glucose uptake exhibited a pronounced maximum around pH 6.0. The time curve of uptake did not seem to be altered by pH, that is, constant levels of D-glucose were always reached by 5 min incubation at 25° C. The absolute amounts of 'extra' uptake varied between 0.3 and 1.0 nmol per mg membrane protein (measured at 1 mM D-glucose) in different preparations. Bacterial contamination is not responsible for our observation.

To determine whether the 'extra' Dglucose uptake represented binding to' the brush border membrane or transport. into the intravesicular membrane space we measured the dependence of D- and L-glucose uptake on the osmolarity of the suspension medium at pH 7.5 and pH 6.0 (Fig. 1). D- and L-glucose uptake at pH 7.5 and also L-glucose uptake atpH 6.0 can be accounted for totally by transport into an osmotically active space (line goes through the origin). The 'extra' D-glucose uptake at pH 6.0, however, is independent of the medium osmolarity suggesting binding (300 pmol per mg protein in Fig. 1; intersection of the line with the ordinate).

The rate of p-glucose transport was found to be equally fast at all pH values between pH 8.0 and 5.0. That transport can proceed without measurable binding, at least on the scale indicated in Fig. 1, suggests to us that transport and measured binding are unrelated. At the moment, we have no hint as to function or origin of the p-glucose binding.

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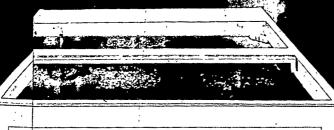
DR FAUST REPLIES-Since brush border region of the intesmucosal cell is a digestive-absorptiganelle, the pumping of monosacles and amino acids across this enrganelle must be considered in the mism by which these substrates are mulated against their concentrationadients within the cytoplasm.

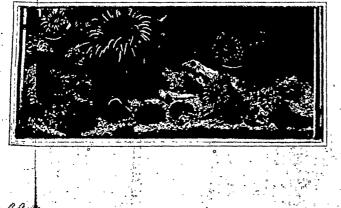
The phenomenon that fer and Sigrist-Nelson have obserconcerns the movement of p-glucoross one section of the structurally dex brush border, the plasma membrie agree that their observed p-glucoinding is not related to transport an feel that it could be caused by bindirdisaccharidases in the plasma mane. Our binding protein, however, ocated in the core fraction of dised brush borders which does not cin hydrolytic enzymes. It possessees for the Na+-dependent trapping specific monosaccharides and amacids that are derived either indire from the hydrolysis of dietary oligostarides and polypeptides or directly m dietary monomers in the intestinumen. We have shown that the specty for this binding is similar to that the active transport of these substancy the small intestine. Furthermore, the servations could not be attributed to terial contamination.

We visualise that our isled binding protein is involved in the 1-dependent active transport of sugaand amino acids from the core region the brush border into the cytoplasnThis occurs after the initial penetration hese monomers through the plasma embrane.

We concede, however, this protein can only be regarded proionally as a transport protein until binding is adequately studied. Debling of the sugar and amino acid terry complexes could be triggered by a offormational change in the binding pron caused by the low intracellular Na+ incentration, the replacement of Na+ the ternary complex by K+ from the gh K+ intracellular region, and by a imbination of these phenomena with theid of energy derived directly from cellul metabolism. None of these conditions vas prevalent in our Sephadex G-75 fractionation therefore D-14 glucose and studies. L-14C-histidine remained tihtly bound to the isolated protein subuits.

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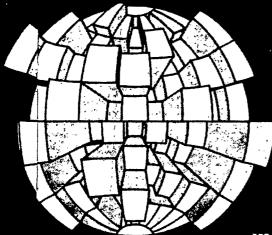
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Earthquakes are the most devastating natural phenomenon known to man and have taken an enormous toll of life and property throughout human history. Until now, we have had no accurate means of predicting their occurrence although much effort has been put into research to discover the possible mechanisms which trigger off these cataclysmic events. John Gribbin and Stephen Plagemann present in this book a new model which aims at predicting earthquakes through the study of the hitherto unrecognized and apparently unrelated phenomena of planetary alignments, sun spot activity and the solar wind, and their vital role in determining the course of natural events on earth. These causal links are shown to affect earth movements in active seismic areas and thus unleash the powerful forces which areas and thus unleash the powerful forces which cause earthquakes.

But their research goes beyond the general model. A particularly unusual planetary alignment will occur within the next articularly unusual planetary alignment will occur within the nexten years and the authors argue a convincing scientific case for major earthquake activity during that period and stipulate the year in question. In particular they show that an earthquake of devastating proportions may well occur along the highly active seismic zone of the San Andreas Fault in California which runs through the cities of Los Angeles and San Francisco. The physical, social and economic implications of a major earthquake in this populous area are far-reaching. They make this a book which should be read by all scientists and non-scientists concerned about the effects of natural and non-scientists concerned about the effects of natural events beyond the immediate control of man.

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## CHARLES C THOMAS - PUBLISHER

HUMAN AND ECOLOGIC EFFECTS OF NUCLEAR POWER PLANTS edited by Leonard A. Sagan, Palo Alto Medical Clinic, Palo Alto, California. Introduction by Rolf Eliassen. (15 Contributors) There is a widespread need and public desire to better understand nuclear power and its consequences. Reactor technology and its ecologic effects often exceed the detailed understanding of many experts. Although the book is directed toward an exposition of human and ecologic effects, the first section details reactor design and engineering. Such material is necessarily included since many readers will be unfamiliar with reactor design and should have available some understanding of reactors, their operation and methods of generating and releasing radioactivity. Public attention has been focused on nuclear power because of the concern for the environment which has suffered from past and present technology. Contamination of air and water with reactor-produced radioactivity has aroused fears of radiation hazards to both persons and the environment. The mood of the public has generated the desire for wider participation in decision making and the loss of faith in those institutions to which these decisions have traditionally been delegated. '74, 560 : pp. (7 x 10), 138 il., 86 tables, \$34.50

FISH CHROMOSOME METHODOLOGY, by Thomas E. Denton, Samford: Univ., Birmingham, Alabama. This practical guide to the identification of fish chromosomes and techniques for their study is written for beginning and veteran investigators interested in the cytogenetics of fishes. Beginning with an introduction to the classification and maintenance of fishes, the author shows how to obtain and present chromosome data, provides a checklist of chromosomes containing the numbers and karyotypes from over 500 fishes, and presents a current analysis of the fish karyotype. The techniques presented include primary tissue cultures, leucocyte cultures, and working with epithelium from fins, scales, gills and internal organs. Advantages and disadvantages of each technique are given. '73 176 pp., 10 il., 1 table, \$11.50

LECTURES OF THE PHENOMENA OF LIFE COMMON TO ANIMALS AND PLANTS, Volume I by Claude Bernard. Translated by Hebbel E. Hoff, Roger Guillemin and Lucienne Guillemin, all of Baylor College of Medicine, Houston. Bernard's work shows the necessity of knowing objectively the invariable elementary properties which are the fundamental basis of all the manifestations • of life. This is the goal that the general physiology proposes for itself. Life lies truly within the organic elements; it is here that the real physiological problems should be placed. In this volume Claude Bernard has summarized the whole of his doctrines, and it is the most complete and the most systematic work that he leaves to the scientific world. This book is in reality a program for general physiology compiled in 1878 from nine lectures delivered at the Museum d'Histoire Naturelle, Paris, France. An excellent historical account of the work of one of the 19th century's most prominent physiologists. '74, 336 pp., 80 il., \$12.95

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# reviews

THE focusearch into the process of technol innovations seems to have shifteently to the developing countries. the problems of 'high' technologyscience-based industries. one now hincreasingly about transferring teogy to the countries of the Third Y; and it is not advanced technology intermediate, or other appropriatchnology. This development in tanguage of technology transfer re; the simple fact that innovation i complex social (some would say cal) process and that the experience he western nations with technologiclevelopments will not transplant & to other countries trying to make transition to industrialisation.

The expace of western nations over the lavo hundred years forms the historicackdrop to the manifold proble of making and implementing deons intended to bring about modernisation, the aur makes clear, involves the marriagf technology and civic life. By this means that every developing sow must be viewed as a complex of already existing and functioning tial and economic processes and tho decision to introduce new technoly can hope to succeed without an erational understanding of this fact. if to guide the reader in the implicans of these facts, the author painkingly documents the industrial hirry of the western world

## **Industrial** revolutions

M. Gibbons

Technology and Civic Life: Making and Implementing Development Decisions. By J. D. Mongomery. Pp. 239. (MIT: London and Cambridge, 1974.) \$12.50.

and points out the relevance of certain developments in the contemporary context of developing countries.

A recurring theme in the book is the changing relationships among three modes of development politics; national planning, local options and individual choice—as forces that can direct the use of technology for advancing the quality of life in the undeveloped countries. This theme also provides the introduction to the theoretical structure of the work-a structure, it seems, that is deeply rooted in a behaviouralistic theory of politics. It is important to remember, cautions the author, that technological change implies changes in behaviour and that these cannot be brought about edict or fiat but must begin with a thorough grasp of the attitudinal status quo and build from that basis. But the modernisation of behaviour also cannot ignore the politics and structure of the community at the local level because individuals form an integral part of this structure and constitute its 'politics'. By the same

token, modernisation cannot be achieved in any significant way by the transformation of the community at the local level alone. Because technological development requires the reorientation of all communities more or less simultaneously, unless it is directed at the national level modernisation will be a fragmented affair. Thus, the characterisation of the developmental problems is, in terms of these levels, intimately linked together. Accordingly, there are also three 'analytical orders' or levels of decision to be considered in applying technology to social purposes. The first order decisions are those which define and develop programmes for the purpose of benefiting identified elements of the society. The second are those that identify the administrative organisation or combinations of institutions that are to be assigned the role of moderniser and change agent. The third prescribes the incentive system by which administrators are to elicit modernising responses from citizens, participating in the programme.

Because of its tight organisation and conceptual sophistication the book makes interesting and stimulating reading. Further, because even in advanced societies there are sectors which are becoming modernised or in need of becoming modernised, there is much in the way of insight and common sense for those in charge of regional development policies.

SPEAKING alne with a personal interest in the engiring applications of holography, I find reading this book to be a refreshinexperience. In addition to contributing ersonally, the editor has gathered cdributions from 14 other internationaly recognised experts in fields of optal measurement or display. Although hography features prominently the bok is by no means confined to that topi nor is it just about nondestructive sting. The title is a bit artificial ancseems almost to have been chosen as a excuse to bring this work together. In pite of that, the aspects of nondestructie testing do form a thread of continuitythrough a work on optical measuring tehniques which are biased heavily to thise which use lasers and to a lesser extert to those using coherent radiation in the form of microwaves or ultrasonic

## **Optical measuring** techniques

John N. Butters

Holographic Nondestructive Testing. Edited by R. K. Erf. Pp. xvii+442. (Academic Press: New York and London, July 1974.) \$28.00; £14.00.

coherent radiation for measurement or testing would do well to read this book. It contains descriptions of practical test layouts and a wealth of experimental 'tips' which help to maximise the usefulness of experiments from a data retrieval point of view. Typical examples are given but some readers will be disappointed to find that these are based in the main on laboratory experiments. Contributions are sectionalised by the author and fall into two categories: substantive material which Any would be user of techniques of forms the core of the book; and reports University, to mention but two.

on significant developments. Sections in the first category contain mathematical backgrounds to the topics covered but there are some abrupt changes in style and technique. Presentation ranges from the quotation of relevant formula to fairly complex derivations extending to. two or three pages. Readers wishing to gain benefit from the theoretical aspects would need a grounding in advanced mathematics.

All in all this is an excellent book for the serious user; it is well referenced and obviously emerges from a group well informed and well practised in their technology. Cited work is mainly of American origin so it may be appropriate to note that the techniques described are available in the UK at centres such as the National Physical Laboratory and the Mechanical Engineering laser facility at Loughborough

## Nuclear techniques

Techniques in Nuclear Physics. By J. B. A. England. Part 1, Pp. viii + 1 - 312; Part 2, Pp. viii + 313 -697. (Macmillan: London and Basingstoke); (Halsted Press, distributors, USA, July 1974.) £10.00 boards; £4.50 paper.

In recent years a wide variety of experimental techniques has been developed which allow measurements of increasing sophistication on the products of nuclear reactions. The techniques are well known to a few specialists but descriptions of them are often only available in scattered articles in specialised journals. The two volumes by Dr England thus fill a real need by providing a comprehensive summary of the more important techniques in a readily accessible form.

The first chapter is devoted to detectors and covers the photographic method, solid dielectric track detectors, gas ionisation chambers. scintillation and semiconductor detectors, spark chambers and Cerenkov and neutron detectors. There follows an account of instrumentation in general, including particle beam detection. measurement and handling, scattering chambers, electronics and on-line computers. The final chapter of Part 1 summarises various types of accelerators: the Cockcroft-Walton voltage doubler, the dynamitron, the Van der Graaf generator, insulating core transformers, linear accelerators, cyclotrons and cyclograffs.

The second volume covers magnetic spectrometers and spectrographs, and particle identification techniques. Among the latter are time-of-flight techniques, techniques of single detector identification by telescope, and problems associated with passing detectors. Then comes a summary of coincidence measurements, angular correlations and lifetime measurements with accounts of the general theory of angular correlations, gamma-gamma and particle-gamma techniques, and correlations with oriented nuclei and huclear lifetimes. The final chapter is devoted to polarised beams and polarised targets.

So numerous are the techniques today that even in a pair of volumes of some 700 pages it has been necessary to restrict the coverage by omitting the Mössbauer techniques, the techniques of beam-foil spectroscopy and the study of the capture  $\gamma$  rays produced by the interaction of polarised thermal neutrons. In other cases where good review articles are available, as in the subject of angular correlations, the discussion has been curtailed accordingly.

The level of the discussion is suitable for senior undergraduates and for first and second year graduate students, and gives a clear account of the basic. physics underlying each technique, together with many references to papers and monographs in which more detailed accounts can be found. There is a comprehensive list of references at the end of each chapter but, unfortunately, no alphabetical list of authors. The subject index is inadequate, so that it could take some time to find the account of a particular subject.

P. E. Hodgson



Taken from the frontispiece of the Atlas of Human Anatomy. Ninth English edition. By Frank H. J. Figge. (Original author: Johannes Sobotta.) Vol. 1: Regions, Bones, Ligaments, Joints and Muscles. Pp. xv+275. Vol 2: Visceral Anatomy. Pp. xii+247. Vol. 3: Central Nervous System, Autonomic Nervous System, Sense Organs and Skin, Peripheral Nerves and Vessels. Pp. xii+354. (Urban and Schwarzenberg: Munich, Berlin, and Vienna, 1974.) No price.

## **Energy spectrum**

Energy: Demand, Conservation and Institutional Problems. Edited by Michael S. Macrakis. Pp. xxvii+556. (MIT Press: Cambridge and London, 1974.) \$25.00.

To undertake the comprehensive reporting of 38 conference articles requires dedication, particularly when the modelling of energy systems is the subject of a major part of the work. The fact that Michael S. Macrakis achieved this objective in a hard backed volume of 530 pages within about a year is noteworthy.

The editor can be excused for rele-

gating another 28 article stract form, to an appendix foons of space, delayed submissionecause they were "addressed to stergy". To add variety and intere best of the articles on sunshind have been included in full and mewhat heavy going sections on ate and disaggregate modelling cove been reduced without great los

The sections of the behich include supply and demannsportation, and conservation, p: helpful subdivisions for experts wish to confine their reading. Ehe energetics enthusiast would however, be impelled to read thok from cover to cover as it m periodic random selection of a fessages for

short perusal.

The reader interested ir prospects and problems facing a wtrying to supply future energy need find half a dozen relevant pap Another dozen contain useful data can thus reduce searches elsewh A great deal of the data and submatter is, however, of relevance ly to the United States.

Readers of this volumill quickly appreciate that national tern in the United States is having aitive effect.

Improved methods fonergy production and applicationnning have been developed. There, a greater awareness of the fac affecting energy economics and thterrelation of the subject with envimental and real resource costs. The is also an appreciation of the neeor a wider variety and depth in rgy assessments, and there has be more concentrated research to irove energy technology.

It was good to note the few European authors were able contribute to the conference. A teafrom Queen Mary College, Londo were very active in providing so interesting papers on world energyodelling, the implications of nation policies on world energy, oil transpation studies, energy economics and atmospheric pollution. Several of the deal with components of the mathatical model being built up by the Ergy Research Unit at Queen Mary ollege, which should become an inchsingly useful tool for policy assistane in Britain.

But there are many petical technological problems to I solved and policy decisions to be nde before the editor's introductory cestion is answered for each coutry operating within its own uniqe limitations. "How quickly", he ass, "with what set of energy sources and at what prices can a supply of energy be secured, which satisfies environmental and safety constraints thile accommodating prescribed econonic paths?"

G R. Bainbridge

1974.) \$10.25.

is well isted with 16 figures, 9 of be enormous. which aptographs; and there are 359 refes for further reading.

The og chapters are concerned with theile male technique, still the only tic control method with a claim ccess (albeit temporary). ample, Ilillion flies a week make interestinading. Radiation, chemosterilants hybridisation as methods for produsterile insects are covered in detail here is no comparison of their relamerits. The more sophisticated ge approaches proposed for controllinects, such as chromosome manipular are thoroughly covered, and althi much laboratory research is g devoted to them, field trials havet been too promising.

There tant encouragement here for thoseho incline to the view that gene methods will solve our pest probs and that the end of insecticide nigh.

Althoughere is little discussion of future pectations for these approaches pest and vector control, the book as a succinct coverage of the subject date with, perhaps, a bias towal medically important insects, in rticular, mosquitoes; no doubt a lection of the author's experienced interests. I. Maudlin

Biological ntrol by Natural Enemies. By Paul Dach Pp. 323. (Cambridge University Press: London, £5.50; \$14.

This is a mely and valuable book, written in haracteristic style by one of the works leading biological control worker It highlights the disasters that have flowed the heavy use of broad-spect|m pesticides and emphasises the highly advantageous costbenefit ratioresulting from successful biological ontrol projects. Numerous historical alunts of successful or partially successful campaigns are given, which highlight the colossal difficulties the early wrkers laboured under in and intuition as well as sound scientific understanding, in searching out the native range of natural enemies.

DeBach rihtly emphasises the crucial importance of correct taxonomy and of seeking gographical races that are

G. Dav Pp. ix+158. (Academic ploration for natural enemies has not Press: h and New York, August yet been attempted for more than 5% of the world's pests and it is certain This brovides a useful overall that an even lower percentage of weeds should study. statemel current and proposed has been considered for attack by this methodshe autogenous control of means. The potential of classical bioagricultud public health pests. It logical control therefore continues to

DeBach makes a point that will bear emphasis: "How to put everything together to choose a potentially effective enemy beforehand with certainty is still beyond our grasp"; and further "... the natural enemy ... considered The tecles for rearing, for ex- to be best often turned out to be inconsequential and, in fact, the best one even may be overlooked for years, as was Cyrtorhinus, the egg predator of the sugar-cane leafhopper." Although attempts to assess in advance the potential of biological control agents must

## More campaigns in the pest war

continue (and they are gradually providing a basis for sounder choices), there is still no promise of a method for accurately forecasting the impact of a particular species. One could therefore join issue with Professor DeBach when he quotes liberally from Alvah Peterson in support of a plea for the study of the 99% of insects that are unimportant to mankind. There is not a shadow of doubt that biological control would languish indefinitely if significant proportions of its rather limited manpower and resources were diverted for this purpose. In this context it is fortunate that there is little evidence that the presence of relatively ineffective introduced enemies hampers the activities of effective agents.

In addition to the great economic potential remaining in 'classical' biological control there are also prospects for the artificial augmentation of populations of natural enemies in relation to certain problems, as when parasites and predators are bred commercially in large numbers for pest control in orchards, poultry houses, dairies and feedlots.

The author also deals briefly with the absence of global air transport, and other non-chemical forms of pest conthe importance of intelligent sleuthing trol, such as the use of resistant plants, cultural and genetic methods, and pheromones, and he gives some useful discussion on integrated control and on the intelligent and selective use of pesticides where that is unavoidable.

This book is obviously not intended adapted to ill the significant climatic as a comprehensive treatise on biologiand ecological situations occupied by cal control-indeed the vast majority

Genetic of Insect Pests. By the pest. He points out that foreign ex- of references are to American publications—but it gives a generally useful philosophy on applied entomology which all interested research workers D. F. Waterhouse

> The Biochemical Mode of Action of Pesticides. By J. R. Corbett. Pp. ix+330. (Academic: London and New York, 1974.) £7.20; \$18.50.

> Any author attempting to describe what is known about the mode of action of poisons faces an unenviable yet stimulating task, for information on the molecular basis of normal physiological activities is frequently incomplete. Impulses pass along nerves by the opening of ionic 'gates' but little is known of the mechanism operating those gates. The same applies to the coupling of electron transport with phosphorylation in mitochondria and chloroplasts, to the biochemical basis of auxin action, and to the mechanism of oxygen release in the second light reaction of photosynthesis.

> As a majority of pesticides probably affect such processes, any account of their action must, of necessity, be incomplete. Dr Corbett is to be congratulated on one of the best attempts so far made to collate available evidence and to present it in a way comprehensible to all biologists possessing a background knowledge of biochemistry and supporting chemistry. It is a valuable reference book for the undergraduate library collection but is not likely to be recommended for private purchase by students of agriculture or horticulture.

> The book comprises three main sections covering compounds acting respectively, on photosynthesis, nerve action and growth. They include tables of pesticides, which are valuable sources of information. The tables would have been even more useful, however, if some indication could have been given of the relative practical importance of the hundreds of compounds they con-

> An author tends to provide space according to the information available on a group of compounds, rather than in proportion to economical importance. Dr Corbett cannot, therefore, be criticised seriously for the fact that some 30 pages are devoted to the useful but locally important urea and triazine weedkillers whereas the crucially important phenoxyalkanoic acids are dismissed in five text pages. Similarly, although it is grand to encounter an afithor with the courage to face the unpopular truth that DDT (despite its manifold faults) has probably saved more lives than any other chemical made by man, it is a pity that it is dismissed in about eight pages.

K. A. Hassall

#### Bird communities

Competition and the Structure of Bird Communities. By M. L. Cody. Pp. viii+318. (Monographs in Population Biology, No. 7.) (Princeton University: New Jersey, July 1974.) \$6.60 cloth; \$3.65 paper.

Copy's book is of more general interest than the title would indicate for he has attempted, with some success, to effect a synthesis between the data of field biologists and the niche and competition models of theoretical ecologists. It will be of interest even to ecologists who hate birds. Cody emphasises the importance of "natural experiments" in understanding community structure, and he makes informed comparisons of mainland and island bird communities, and of California chaparral and Chile matorral bird communities.

He presents a great deal of data that are consistent with the predictions of the theory of community structure developed by the late Robert MacArthur. For example, Cody finds that variation in foliage height diversity accounts for 81% of the variation in bird species diversity in eight North American bird communities (page 128). Cody's extensive field data point out some of the weaknesses of current theory: suppose that one can measure accurately the amount of competition for food, and that one can measure accurately the amount of competition for habitat. How can they be combined into one number that reflects the total amount of competition between two species? If competition along different niche axes (food, habitat, and so on) is independent, then the total amount of competition is the product of the competition coefficients along the different axes. This assumption of independence has usually been made by theoretical ecologists, but Cody found that addition, rather than multiplication of the competition coefficients along the different axes made for better predictions of community structure.

· At times I found the book frustrating because Cody did not develop some of his insights more thoroughly. For example, Cody repeats and partially justifies one sacred cow of ecology-'jack-of-all trades and master of none' (page 55), but on page 68 he shows that species that occupy a broad habitat range often eat a broad range of foods, and argues that this is optimal. Such a species would seem to be a 'jack-of-all trades and master of some'. Another example is his demonstration of character convergence, which he argues is a consequence of interspecific competition. Yet he does not discuss or test the model of MacArthur and Levins that predicts character convergence when a species invades a community in which resource overlap between species is high. Instead, Cody discusses the relationship with interspecific territoriality, but this seems to confuse cause and effect.

Readers not convinced that competition is a major factor in community organisation may not be persuaded by Cody's book. In some cases, alternative explanations are available for the phenomena Cody attributes to competition: for example, geographical separation of closely related species. Nevertheless, Cody presents a large diverse body of data consistent with competition theory. Any refutation of the importance of competition in determining community structure must; account for the extensive observations David C. Culver Cody has made.

## Carbon and light

Organic Photochemistry. By J. M. Coxon and B. Halton. (Cambridge Chemistry Texts). Pp. vii+196. (Cambridge University: London, 1974.) £4.20; \$13.00.

ALTHOUGH several new books on organic photochemistry, aimed mainly at senior undergraduate and graduate students, have been published in the last two or three years the subject is undergoing such rapid expansion that new titles are always welcome. The book under review is divided into five chapters dealing with an introduction to photochemistry; intramolecular reactions of olefinic bonds; intramolecular reactions of carbonyl compounds; cycloaddition reactions; and substitution, oxidation, and reduction. The text is commendably free from typographical errors.

One of the many problems confronting authors in the area of organic photochemistry is the quantity of scientific literature to be covered. There is no possibility of encyclopaedic coverage in a small volume but the information cited should at least be correct. Unfortunately, this is not always so in this book. On page 140 it is stated that 2-methoxynaphthalene affords a (2+2) dimer involving the 9,10 bonds, though, in fact, the dimer formed is of the (4+4) type involving addition across the 1,4 sites of the substituted ring. This structure has been verified by X-ray analysis (Chem. Commun., 978; 1969).

On the whole the authors have kept this book up to date although, inevitably, since the publication date new results have out-dated some of the reaction mechanisms. The book is very readable and gives coverage suitable for the students at whom it is aimed.

William Horspool

## Ising model

The Two-Dimensional Isinel. By Barry M. McCoy and Tal Wu. Pp. xvi+418. (Harvard rsity: Cambridge, Massachusetts; d University: London, Febru 1974.) £12.50.

THE two-dimensional Isingl gives a simplified description of; range of physical systems inch fluids, magnets and binary allwithout question it is the most thory investigated model in statisticahanics. Its main interest is that, to simply formulated, it has a surprrichness and complexity, and through light on the behaviour of systemergoing a phase transition. In 194Onsager performed the major feat lculating the partition function or square Ising lattice in zero fieled found some of its thermodynamoperties. The results profoundly iged the theory of phase transitiond critical behaviour.

Onsager's calculation later investigations by others wnotorious for their difficulty, and made the study of the s model practically a branch cheoretical physics by itself. "In 1962 the Dutch physicist P. Kasteleyn achieved considerable sification by showing that the two-dimonal model in zero field is equivalen the combinatorial problem of png dimers on the bonds of the lati such that each lattice point is cold by the end of one dimer, and inting the number of ways in whichis is possible. This problem has mple solution in terms of the Pfaf of a matrix which can be evaltd without much difficulty. Over thet 12 years the method has become awerful tool by which many more deed properties of the model have b evaluated. The authors of the presemonograph have made notable contritions in this field. Even though the mod is simple in principle, research pars still tend to be hard reading becse of their conciseness and the amot of detail. The aim of this book to give an elaborate exposition of the dimer method and its applicati in various situations. It does not psent a comprehensive survey of all t work which has been done on the ty-dimensional Ising model. The auths have succeeded admirably in the limited aim and have given a carefuexposition of the mathematical techniues involved and the results obtained, or those who want to be acquainted ith the model and its history some eisting reviews would seem preferable. But this book is recommended to anyne aspiring to research in the area ad wishing to acquire a working knowledge of the B U. Felderhof dimer method.

up to lith a footnote to each netic rese and infrared spectroscopic teues, with the emphasis this class of compounds. strongly he first of these three. pounds. In the results are briefly interpretee strength of the book is in the its themselves.

The fir apter outlines the general bondproperties of phosphorus and contain essential and salutary analysis de reliance that can be placed on molecular dimensions derived fi X-ray structure determinations.he second chapter is devoted the many structures of phosphorund this complex story, very neat xposed, leads naturally to the nechapter on phosphides, where theructural diagrams will, perhaps iitably, only be easily understoody crystallographers.

compounds which phosphorous is mainly boil to elements in group six, amonghich oxygen predominates. A st survey of oxides, sulphides andlenides is followed by a

compoulat have been reported gantly classified by their structures.

The phosphate esters are covered chapter, in proof, to extend the in chapter 7 which contains a lengthy scope to 1973. The information digression on DNA and is one of the presenteers that derived mainly less satisfactory sections of the book, by X-refraction, nuclear mag- a reflection perhaps of the meagre structural information available for

Chapter 8, on substituted phosphates. The authstricts himself to a com- is lucid and concise and the structures parative sion and establishes the of the isolated molecules that make range of cular dimensions to be up the greater part of this category found wivarious classes of com-lend themselves to the clear repre-

## **Phosphorus** chemistry

T. S. Cameron

The Structural Chemistry of Phosphorus. By D. E. C. Corbridge. Pp. xiii + 542. (Elsevier: Amsterdam, London and New York, 1974) Dfl.250; \$96.20.

sentation they receive. The behaviour of bonds between phosphorus and oxygen atoms is examined in chapter 9 where a discussion on hydrogen bonding is concluded by a summary of P-O bond length (and interbond Chapters 9 (202 pages) cover the angles) over the range of compounds described in the previous five chapters.

Hydrides, nitrides, halides and phosphines are the subject of chapter 10 and the following chapter covers the while still being able to supply single compounds where phosphines act as items of information, in context, with longer discion on orthophosphates. ligands with metal atoms. There, with an evaluation for those with less In these ended structures, as with a vast range of material the author the phospes, the structural dia- has little alternative but to compile high price there will be few research grams are t always easy to inter- a catalogue of the known structures. pret. The coensed phosphates are ex- Details of the ring compounds of phos-

This w a detailed source book amined in chapter 6 where this com- phorus are found in two chapters-12 of the ure of all phosphorus plex series of compounds are ele- and 14—with the first restricted to a simple survey of the structural chemistry of the phosphazines and the second swiftly covering all the remaining ring compounds. Curiously inserted between these two chapters is one on isomerism and optical activity.

The final chapter on cage structures is very short: few containing phosphorus have been reported, and the text is padded somewhat with analogies to cage systems formed by atoms other than phosphorus. There are appendices with a list of unit cell and spacegroup data for phosphorus compounds, an infrared correlation chart and NMR chemical shifts for typical compounds. and 2,649 references.

The book is printed on good quality paper with an elegant typographical balance between text and diagrams. It must have been a proof reading nightmare, but though few errors remain, it is disconcerting to find that a diagram of vitamin B12 (labelled vitamin A) has a structural formula with two very uncanonical carbon atoms.

Throughout the work the author not only exercises a balanced judgement on his source material but presents the evidence on which he bases his judgement. The book will thus be a valuable aid and happy hunting ground for those working in the field of structural chemistry of phosphorus specialised requirements. Despite its laboratories that would want to be without access to this book.

## Crystachemistry

The Major rnary Structural Families. (Crysto Chemistry of Non-Metallic Matals.) By O. Muller and R. Roy. Pp. +487. (Springer-Verlag: Berlin and Iw York, 1974.) DM76; \$31.10.

In his introduion to this first volume in the series of publications on the crystal chemisy of non-metallic materials, Professo Rustum Roy comments on the fact tht few books have been published on the subject of crystal chemistry. If he forthcoming volumes in the series haintain the high standard set by his issue much of the deficit will have been made up.

The introduction is extremely comprehensive and makes certain that the reader understands how to make fullest use of the large amount of information given in the subsequent chapters. Frequently, introductions seem to have been written is an afterthought but here one senses that considerable care has been taken with what should be a most important chapter.

Three major structural families are dealt with in detail: A2BX4, ABX4 and ABX3; with a small number of other structures collected together in table form at the end of the book. Each major structural family is subdivided into individual structural types based on a particular compound. Summaries at the start of each chapter indicate clearly the range of structures covered, thus enabling the reader to locate rapidly any structure of parti-cular interest. Of especial value is the grouping of all the individual structure types into a structural field map at the end of every chapter. The reader can then appreciate to the full the interrelationships of each structure with those of others in the same group. All of the diagrams in the book are of very high quality.

A problem for any author writing on crystal chemistry is how best to present the large amount of crystal data which are required. In this book the authors have chosen to place nearly all such data, in tabular form, into an appendix. Some selected data necessary for an understanding of the text is tabulated in each chapter. The result of this is, however, that almost half of the book is made up of the appendix.

There are two small, but pleasing, aspects of this book: first, the comprehensive and easily found tables of ionic radii, which may well be consulted more frequently than many of the tables; and second, the short sections on the applications of some of the compounds mentioned.

The volume is an excellent reference work and perhaps my only criticism is that the range of structures covered is limited; but one can appreciate that any volume which attempted to cover, in this amount of detail, the whole range of ternary structures would become overlong and unreadable.

M. G. Barker

## Radically speaking

Microwave Spectroscopy of Free Radicals. By Alan Carrington. Pp. ix+264. (Academic: London and New York, June 1974.) £4.80; \$12.50.

This is a highly specialised monograph by one of the major workers in the field discussed. As expected, it is clearly and precisely written and will be invaluable to those interested in, or working in, that field. It is not, however, likely to be of great interest to conventional electron spin resonance (e.s.r.) spectroscopists, nor is it, in any sense, a text on microwave spectroscopy as such.

The 'term 'microwave spectroscopy' is extended to cover all experiments "in which the absorption or emission of microwave radiation by a molecular species can be detected directly or indirectly". The term 'free radical' is extended well beyond the normal doublet state species to include  ${}^{1}\Sigma$ ,  ${}^{1}\Pi$ , <sup>1</sup>Δ, <sup>3</sup>Σ and <sup>3</sup>Π states. Thus, the experimental section includes sections on microwave rotational spectroscopy. gas-phase electron resonance, microwave/optical double resonance, and molecular beam spectroscopy.

Similarly, the 'free radical' species include a wide range of diatomic species such as, CS, A1F, H2, O2, SO, SeO, NF, CN, and the small number of triatomic molecules so far studied, namely NCO, NCS, HNO, CF2, SiF2, HCO, DCO, NO<sub>2</sub> C1O<sub>2</sub>.

The author starts by asking if perhaps this specialised field should be viewed as a "backwater": that it should not be is well demonstrated; in this excellent book.

Reactive Free Radicals. By J. M. Hay. Pp. viii+158. (Academic: London and New York, June 1974.) £4.40; \$11.50.

This book should have a far more explicit title such as 'Structure-reactivity relationships for Organic Radicals'. The author is very much concerned with kinetic parameters derived from reactions in the gas phase. I suspect that the treatment is too biased towards the author's particular conceptions to make it useful for undergraduates, but other workers should find it stimulating.

In the preface we are warned that the book is based on views that are "perhaps even naive" and I fear that this may be a valid warning. Nevertheless, I enjoyed reading it and found several suggestions well worth toying with. I must say, however, that I found an awful lot of glib statements to which I would take exception, such as, "charged radicals are normally produced by electron-transfer at electrodes or from charged species". Or we are told that high-energy radiation pro(if that were always true, I would be out of business). Then we are told that electron spin resonance (e.s.r.) spectroscopy will not detect pairs of radicals formed in solvent cages, despite the fact that one of the most important e.s.r. developments in recent years has been the study of such 'triplet' species.

One very misleading suggestion is that the breaking of the C-C bond in R<sub>3</sub>C-CR<sub>3</sub> molecules may result in the formation of σ-radicals R<sub>3</sub>C--sp<sup>3</sup>hybridisation is implied—despite the fact that the author accepts that R<sub>3</sub>C· radicals are planar or very nearly so. Even more unfortunate is the fact that e.s.r. evidence in favour of this idea is cited, although this has long since been refuted. Again, we are told that a proton hyperfine coupling of  $\pm 16$  gauss for HC $\equiv$ C is "hard to reconcile with a linear structure". Why?

Throughout, very simple valencebond structures are presented (often consuming a lot of space) and the results of sophisticated molecular orbital calculations are completely ignored. I can see very little justification for such an approach in a study which is primarily concerned with structural probdems.

This short book in no sense overlaps with the excellent recent work on radical displacements by Ingold and Roberts, despite the title. The presentation is satisfactory except that references are given at the end of each of the five chapters. As no help is given to the reader, one inevitably turns up the wrong set of references which can prove to be very misleading. Please can we have all references at M. C. R. Symons the end?

## **Nervous systematics**

Evolution of the Nervous System. By Harvey B. Sarnat, and Martin G. Netsky. Pp. xvii+125. (Oxford University: London and New York, September 1974.) £5.75 boards; £4.00 paper.

This is one of the very few comparative anatomy textbooks of reasonable textbook size. It explores neuroanatomy, system by system, in a functional way "through the dimension of time as revealed by analysis of evolution". The book starts with a general overview of vertebrate evolution, and then proceeds with a system by system analysis, including the brain, spinal cord, and the autonomic nervous system. The emphasis is always on how the evolutionary series relates to the structural and functional organisation of the human brain. The book does not cover invertebrate nervous systems, nor those aspects of vertebrate evolution which are divergent from the point of view of the human nervous system. Written by

duces an "embarrassment of products", a neurologist and a neurologist, the text brings out wherossible the bearing of the conve approach on our understandvarious. congenital and pathologiaditions in the human nervous s as encountered in clinical p. The authors do not hesitate al with provocative and speculatints, although always making it where they are being speculativ

There is an extensive bhy, and the references are reast up to date in the majority of sections. The text, however, lacferences to recent developments ie study of central biogenic amirspecially in the accounts of the looeruleus, hypothalamus, and nigroe system. There is an interesting af selected brain sections in differencies.

This comparative panatomy textbook is neatly an indsomely bound, and moderatelyced. The text is highly readable as all the basic anatomical arranges are described, it is complete in and does not require additional ence to a standard neuroanatomy The chapters are well indexed, arithin each chapter the sections are rly headed and subdivided. This me it easy to look up any particulaystem, and enhances the value ole book as G. Raisman a reference work.

## Tropical fores

Tropical Forest and its vironment. (Tropical Ecology Seric By K. A. Longman and  $\overline{J}$ . JeniPp. x+196. (Longman: London, Jui974.) £1.95.

This lucidly written andeful booklet belies its ambitious titler, as stated in its preface its principontribution is a synthesis of results ohysiological experiments on Ghanai forest trees carried out in controlleavironments, and parallel field investions; both are then related to sting knowledge of rain forest ecoly. The careful accounts of the autho experiments provide new and reward insight into West African rain fores but attempts to relate them to fores elsewhere in the tropics, and more rticularly the additional general chapis on subjects unrelated to the experients, are of variable quality, as th authors too often assume, wrong that West African forest condition are universal in the humid tropics, he account of tropical forest soil, for istance, is misleadingly over generased; that on biotic factors is so suerficial that it would have been bette omitted; previous data is somethes misquoted (notably in Fig. 4.9); a'd much recent literature is left unconsisted. But there are well designed diagrams and 25 pages P. S. Ashton of photographs.

# obituary

#### M. Ewing

MAURICE EWING, the eminent oceanographer, died in Galveston, Texas, on May 4, 1974.

Ewing attended the Rice Institute (now Rice University) for both his undergraduate and graduate education in Physics. On receiving a Ph.D. in 1931, he was appointed to the Physics Department at the University of Pittsburgh, then at Lehigh University where William Bowie, Richard Field and Walter Bucher introduced him to the problems of seagoing geophysics. Using equipment and help from F. A. Vening Meinesz, pendulum gravity measurements at sea were begun. With his students (and lifelong associates) Vine. Webster, Woolard and Worzel, techniques were developed for making seismic measurements with the explosive source and automatic recording equipment on the seafloor. In this manner, the first marine seismic refraction measurements were attained just before the outbreak of the Second World War. Sediments were found to be thicker over the continental shelf than in the deep sea.

In 1940 Ewing took leave of absence (he was then an Associate Professor of Geology at Lehigh) to move with his group to the Woods Hole Oceanographic Institution and work problems of submarine detection in association with Columbus Iselin, who had become impressed with the decisive role played by the thermocline in determining sound transmission characteristics in the upper ocean. Ewing and Vine adapted the Sphilhaus bathythermograph for use on vessels while underway. Iselin, Ewing and Worzel produced a manual, Sound Transmission in Sea Water for use in a basic training course at Woods Hole for Naval Officers. Ewing and Pikeris were the first to recognise the importance of the minimum in soun velocity for long-range sound transmission (SOFAR). The expected waveguide effect was demonstrated with a dramatic test: a small explosive was etonated at depth in the eastern Atlantc, and recorded on the western side with hydrophones suspended into the SOFAR channel. Ocean bottom photography, developed as a sideline n previous years, was applied to undewater shipwreck photography. With some incredible luck, Ewing obtained a picture of a wreck's nameplate; he would carry this in his

pocket to convince sceptical Naval officers of the usefulness of underwater photography for identification purposes. The first seabottom wave recorder was built by Ewing.

In 1946 Ewing and some of his group returned to academic work-this time at Columbia University, where they soon founded the Lamont Geological Observatory (now Lamont-Doherty). Ewing served as Associate Professor of Geology, then Professor, occupying the Higgins Chair from 1959 to 1972. Under Ewing's direction, the Lamont Observatory moved into the forefront of geophysics and geology, especially as related to the oceans. Seismic refraction measurements were resumed, this time not with bottom-dropped instruments but with suspended hydrophones. Ewing developed and used towed magnetometers (first flux-gate, later nuclear precession), and initiated continuous reflection profiling with air-gun sources. He improved techniques for precision depth recording, gravity measurements (from submarines and later from surface vessels) and long piston coring, and carried out heat-flow measurements in conjunction with piston coring.

Yet another contribution was the suggestion that turbidity currents played a vital role in sedimentary processes. The study of the sedimentary evidence led to a theory (with Donn) for climatic change.

In association with Frank Press and Wenceslas Jardetsky, Ewing launched a major era of seismology with the study of surface wave dispersion, explaining many previously misunderstood features of seismograms. The book Elastic Waves in Layered Media by Ewing, Jardetsky and Press served as a basic text for a new generation of seismologists.

Thus. Ewing's career was one of many thrusts into the unknowns of the Earth: the Continental Shelf, submarine ridges and trenches, the structure of continents, ocean basins and the earth as a whole, the structure and origin of sediments and the structure of the oceanic water column. He certainly provided plenty of stimulating competition for other oceanographic laboratories.

The development of the theory of ocean floor spreading and plate tectonics was made possible to a great extent by the observations made by Ewing and his coworkers. The dis-

covery of the thinness of the oceanic crust and oceanic sediments and the discovery of the continuity of the midoceanic ridge system were especially important keys. Ewing enthusiastically supported the JOIDES drilling programme which was to give dramatic confirmation to the concept of ocean floor spreading. He maintained a healthy scepticism for any new schemes proposed either by himself or others and the theory of the new global tectonics was no exception. He was later to comment that he was amazed to see so many of the facts that had puzzled him about the oceans explained by such a simple theory.

Ewing was a man of quiet courtesy, which belied a singlemindedness of purpose. Once the priorities were set, nothing would be permitted to get in the way. Thus, he would turn the lathe in the ship's crammed machine shop all night to replace a faulty gear. Once when swinging gravity pendulums aboard submarines, he told one of us: "If we get sub time, no matter where or when, Worzel or I will be aboard" While securing some explosives aboard the VEMA during an early morning gale, Ewing and two shipmates were washed overboard. Ewing and one man were miraculously saved but Ewing sustained injuries from which he never quite recovered. In reflecting upon his own reactions, Ewing characteristically assigned his priority: to stay affoat.

During his long and productive career, he helped train more than 200 graduate students. Affectionately known to his students as 'Doc', he expected no less from them than the hard work and devotion that he himself was always ready to give. His students and colleagues will remember the many times they stepped from his office inspired. and challenged by either a few minutes or a few hours of intense interaction. He was the author of more than 300 papers and many honours came to him: among them, election to the National Academy of Sciences in 1948, the Vetlesen Prize in 1960, Foreign Membership of the Royal Society in 1972. and also the National Medal of Science in 1973.

His last two years were spent very happily as Green Professor of Marine Sciences and Founding Chief of the Earth and Planetary Sciences Division of the University of Texas Marine Biomedical Institute in Galveston.

## **Announcements**

#### Awards

Philip S. Corbet has been awarded the Gold Medal Award of the Entomological Society of Canada.

The Fondation de Physiopathologie Professeur Lucien Dautrebande Prize has been awarded to Professor Durrer for his contribution to clinical and electrophysiological knowledge of the heart

Judah H. Quastel, Hans J. Müller-Eberhard, Hector F. DeLuca, Roger Guillemin, Andrew V. Schally, David Baltimore and Howard M. Temin are recipients of the 17th Annual Gairdner Foundation Awards.

Robert Allan Smith has been made an honorary Commander of the Order of St:Olav, the Royal Norwegian order of chivalry.

#### **Appointments**

James Barron Bridges has been appointed to a second chair of physiology at the Queen's University of Belfast.

Philip John Randle has been appointed to the chair of clinical biochemistry at the University of Oxford.

Malcolm Rowland has been appointed to the additional chair of pharmacy at the University of Manchester.

#### Miscellaneous

The Pharmaceutical Manufacturers' Association Foundation Medical Student Fellowships. Four one-year grants for the academic year beginning July 1, 1975, will be awarded to candidates interested in research and teaching careers in pharmacology/clinical pharmacology.

The Foundation will also award twoyear postdoctoral fellowships in pharmacology/morphology. For information contact: Thomas E. Hanrahan, Pharmaceutical Manufacturers' Association Foundation, 1155 Fifteenth Street, N.W., Washington DC 20005.

Maltwood Fund for Archaeological Research in Somerset. The Royal Society of Arts invites applications for grants in aid of archaeological research to be carried out in the county of Somerset during 1975. For further information contact: J. S. Skidmore, Royal Society of Arts, John Adam Street, Adelphi, London WC2 6EZ.

#### International meetings

December 9, Imaging by Holography, London (R. J.; Neville, Research Division, Kodak Ltd, Headstone Lane, Wealdstone, Middlesex).

December 11-13, Surface Analysis, Slough (D. Nicholas, Head of Analytical Services, Fulmer Research Institute Ltd, Stoke Poges, Slough SL2 4QD).

December 22, Ethics in an Age of Pervasive Technology, Israel (Dr Mordechai Levy, Senate Building, Technion Haifa, Israel).

January 3, Elucidation of Important Mechanisms for Controlling Cellular Integrity, London (Dr D. J. G. Davies, School of Pharmacy and Pharmacology, Claverton Down, Bath BA2 7AY).

January 6-8, The Mechanics and Physics of Fracture, Cambridge (Meetings Office, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

January 6-9, Geophysics and Cosmology of Solar System Observables, (Dr Newcastle-upon-Tyne F Stephenson, School of Physics, University, Newcastle-upon-Tyne NE1 7RU).

January 10, Phase Equilibria and Phase Separation in Polymer Systems, Essex (Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X-8QX).

January 21-22, Power Plants and Future Fuels, London (The Secretary, The Institution of Mechanical Engineers, 1 Birdcage Walk, London WC2R 3LF).

#### Reports and publications

#### Great Britain

Maritime Monographs and Reports. No. 12—1974. Problems of Medicine at Sea. Pp. 35. (London: National Maritime Museum, 1974.)
The Ecology of Human Communities. (An Inaugural Lecture delivered in the University of Birmingham on 21st March, 1974.) By Rowland Moss. Pp. 20. (Birmingham: University of Birmingham, 1974.) 25p. [39]
A Legacy from the Blighted Brain. (An Inaugural Lecture delivered in the University of Birmingham on 7th December, 1971.) By W. Thomas Smith. Pp. 23. (Birmingham: University of Birmingham, 1974.) 25p. [39]
Department of the Environment. Emergencies Arising

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Department of the Environment. Emergencies Arising From Chemicals and Other Substances Washed Ashore. Pp. 11. (London: HMSO, 1974.) 14p.
The Ordnance Survey. Annual Report 1973-74.
Pp. 13 + 5 plates. (London: HMSO, 1974.) 98p. [69]
The Science of the 1st Person. By D. E. Harding.
Pp. 48. (Nacton, Ipswich; Shollond Publications, 1974.)

National Radiological Protection Board. The Work of the NRPB 1970-73. Pp. 66. (London: HMSO, 1974.) £1.00.

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The British Council. Overseas Students in Britain: Statistics 1972-73. Pp. 32. (London: The British Council. September, 1974.) 30p. [129]

An Index of the Living Plant Collections in the British Isles. Compiled by J. T. Williams. Pp. 57. (Kew: Bentham-Moxon Trust, Royal Botanic Gardens, 1974.)

50p

2001; Challenges and Responses. (One-Day Symposium on the Relations Between World Population, Technology, Resources and Business.) Principal Speakers: B. J. A. Hargreaves, D. E. Hughes and E. F. Schumacher. Pp. 40. (Treforest, Mid-Glamorgan: F. C. Slaughter, Glamorgan Polytechnic, 1974.) £1.

The Nuffield Foundation. Report for the Year 1973. (28th Report). Pp. 128. (London: The Nuffield Founda-tion, 1974.)

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The Military Balance 1974/1975. Pp. viii + 104. (London: The International Institute for Strategic Studies, 1974.) £1; \$3. [189]
The Kew Record of Taxonomic Literature Relating to Vascular Plants for 1971. Pp. ix + 394. (Royal Botanic Gardens, Kew.) (London: HMSO, 1974.) £14 net.

Botanic Gardens, Kew.) (London: HMSO, 1974.) £14 net. [189]
British Harvestmen: Arachnida: Opiliones—Keys and Notes for the Identification of the Species. By J. H. P. Sankey and T. H. Savory. (Synopses of the British Fauna, No. 4.) Pp. 75. £1.90; \$5. British Sea Spiders: Arthropoda: Pycnogonida—Keys and Notes for the Identification of the Species. By P. E. King. (Synopses of the British Fauna, No. 5.) Pp. 68. £1.90; \$5. (London and New York: Academic Press, 1974. Published for The Linnean Society of London.) [239]
Freshwater Biological Association. Forty-second Annual Report for the year ended 31st March. 1974. Pp. 210. (Ambleside, Westmorland: Freshwater Biological: Association, 1974.) £1.
Vaccination. Pp. 44. (London: Office of Health Economics, 1974.) 25p.
Annual Report of the Meteorological Office for 1973. Pp. xiv. + 138 + 8 plates. (London: HMSO, 1974.) £1.10 net. [239]
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The Use of the Analmatic System in the Automation of In Vitro. Radio-Isotope Methods. By G. S. Andrews, H. G. Owen and Janet L. Davies. Pp. 16. (Harrow: Searle Instruments, 1974.) [239]
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Royal Institute of Navigation. Annual Report, 1973/1974. Pp. 20. (London: Royal Isstitute of Navigation, 1974.) [259]
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World Health Organization. Tednical Report Series No. 551: WHO Expert Committee on Drug Dependence—Twentieth Report. Pp. B. (Geneva WHO; London: HMSO, 1974.) Sw. fr. 7. [179 Agriculture and Environment, Vol. 1, No. 1, June 1974. Pp. 1-114. 1 volume a year \$\epsilon 4\$ issues. Subscription rate: Dfl. 95; \$36.50, postag included. (Amsterdam: Elsevier Scientific Publiship Company, 1974.)

National Research Council of (mada. Research for Construction. By N. B. Hutcheo (Special Technical Publication No. 2 of the Division (Building Research.) (Ottawa: National Research Couil of Canada, 1974.)

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Zoology Publications from Vioria University

Zoology Publications from Vioria University of Wellington, No. 65: An Evaluatio of an Experimental Model for Sympatric Speciation. I Rosemary Hipkins. Pp. 12. (Wellington, NZ: Depiment of Zoology, Victoria University of Wellingtor!974.) [189 United States Department of thinterior: Geological Survey. Professional Paper 486—: Geohydrology of the Yuma Area, Arizona and (difornia. By F. H. Olmsted, O. J. Loeltz and Burdgeelan. Pp. vii + 227 + 17 plates. (Washington, DC: overnment Printing Office, 1973.) \$11.60. [189 1973 Annual Report of the Prinaceutical Manufacturers Association Foundation Foundation. Pp. 36. (Washington, DC: Pharmaceutical Mafacturers Association Foundation, 1974.) [199 New Zealand. Report of thNational Research Advisory Council for the year end 31st Manch, 1974. Pp. 22. (Wellington: GovernmentPrinter, 1974.) 15c. [209

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Vogelwelt und Landschaftsplaing: Eine Studie aus dem Werdebfelser Land (Bayern Von Einhard Bezzel und Helmut Ranftl. (Tier und mwelt, Neue Folge. Nr. 11/12. (Barmstedt: Verlag Dev Kurth, 1974.) [20]

The Sterile-Insect Technique id its Field Applications. (Proceedings of a Panel onle Practical Use of the Sterile-Male Technique for Inst Control, organised by the Joint FAO/IAEA Divisio of Atomic Energy in Food and Agriculture, and hel in Vienna, 13–17th November, 1972. (Panel Proceedings Series.) Pp. 137. (Vienna: IAEA; London: HMS), 1974.) 128 schillings; £2.90; \$7.

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ADVENTISEI SHOULD BE ADDRESSED TO: T. G. Scéon, Limited, I Clement's Inn, London, W.O. Telephone: 01-242 6264. Telegrams: Tt, London, W.C.2.

#### APPOIENTS VACANT

#### RECTOR

Applications nvited for the position of

DIREKTCES INSTITUTS FUR TIERZUCHND TIERVERHALTEN FORSCIGSANSTALT FUR LAVIRTSCHAFT,

LAVIRTSCHAFT;

a federal researtilution engaged in reproductive biology, arbehaviour and renetics. Staff of 250 includiniscientists. Research budgeted. Facilities: 2,500 f laboratory space; 4 research farms (1200 ha) stock of 1,200 cattle, 2,000 pigs, 1,000 shee/cations: main laboratories and 2 farms at Male near Hannover; laboratory and 2 farms at porst near Lübeck.

Qualifications: mationally established research reputation. Apits with biochemical and physiological balund should be enabled to coordinate the rch programmes of the different sections.

Salary: approx000 DM/annum (B 3 tenure position in govent service).

To apply, serbriculum vitae, summary of research activities of publications and reprints before December 1975 to:

Prof. Dr Immer
Präsident (Forschungslt für Landwirtschaft Bundesalle, D 33 Brauweig Federal Relic of Germany (1792)

(1792)

#### UNIVER Y OF LEICESTER

DEPARENT OF GENETICS (Head of Departn: Professor R. H. Pritchard) Applicatione invited for a post of

#### CTURER

The person anted will participate in the teaching of genetito both medical students and those studying for B.Sc. in Biological Sciences. Candidates must ave a broad knowledge of genetics and prefere will be given to those who combine this withistdoctoral experience in some aspect of nucleic d technology.

Initial salary aciding to qualifications and experience within thrange £2,118 to £4,896 a year plus threshold paients, at present £167.04, plus Superannuation. There particulars from the Registrar, to who applications should be sent quoting reference. G by January 5, 1975. (1986)

#### THE DEPARTENT OF PSYCHOLOGY PRINCETO UNIVERSITY

anticipates several openings for the 1975-76 academic year. On is at the Full Professor level in social psycholog, where we seek an addition to our ongoing pigramme in social psychology. The other(s) at the Assistant Professor level are in general experimental psychology, with concentration in animal behaviour developmental and comparative psychology of personality research. These latter anticipated opinings are not rigidly tied to any specific area of expertises. Sheer excellence in research and teachig will override area-of-interest considerations.

Nominations and applications should be sent to

considerations.

Nominations and applications should be sent to the Chairman, Deprtment of Psychology, Green Hall, Princeton University, Princeton, New Jersey 08540. We are an equal opportunity employer with a vigorous affirmative action programme. (2022)

## **Senior Pharmacologist Anti-Inflammatory Research**

Applications are invited for a senior post in our Pharmacology Department in Welwyn Garden City, Hertfordshire. The department is responsible for carrying our research and screening work aimed at the development of new drugs in which the pharmacologists participate in the research programmes of interdisciplinary groups.

The senior pharmacologist we appoint will be responsible for directing the work of a team of pharmacologists engaged in anti-inflammatory research. Applicants should have a Ph.D. qualification and preferably some post-doctoral experience in anti-inflammatory research.

Our Pharmacology Department is located in new laboratories where the facilities and working conditions are excellent. Conditions of service are above average and in appropriate cases generous assistance with relocation will be available.

Roche Products Limited is part of a major international pharmaceutical firm based in Switzerland and is itself one of the leaders in the industry in the United Kingdom. If you would like to apply for this post please write for further information, and a Company booklet, to the Welwyn Personnel Manager.

Closing date for applications, December 13, 1974.



Roche Products Limited Welwyn Garden City Hertfordshire AL7 3AY

(1896)

#### INSTITUT MAX VON LAUE-PAUL LANGEVIN GRENOBLE—FRANCE

The Institut Max Von Laue-Paul Langevin operates a high flux reactor providing intense beams of neutrons for studies of condensed matter in the fields of physics, chemistry, biology and materials science. Visitors from universities and research centres in the member countries, France, Germany and the U.K., and resident scientists use the high flux beam reactor and the Institut provides scientific and technical support.

Applications are invited for the following post at Grenoble:

### ENGINEER FOR REACTOR OPERATION (MECHANICAL ENGINEERING GROUP)

The successful applicant will be in charge of a group responsible for the maintenance of and adjustments to the mechanical equipment of the reactor and associated facilities.

Applicants should possess a degree or equivalent qualification and have a sound knowledge of reactor operation and of the behaviour of materials subjected to radiation. A working knowledge of French is desirable but not essential, as tuition will be given.

Salary will be according to qualifications, experience and responsibilities. Removal expenses will also be paid and assistance given in seeking accommodation.

Write for application form to: Mr. D. McConville, Science Research Council, c/o British Rail Engineering Ltd., Swindon Works, Swindon, Wiltshire, SN1 5BW, quoting reference and post applied for.

Completed application form should be returned by December 16, 1974. Ref: ILL/05.

#### MEDICAL RESEARCH COUNCIL CLINICAL AND POPULATION CYTOGENETICS UNIT SCIENTIST

A vacancy exists in the Experimental Studies Section of the above Unit for a research scientist, who will be principally concerned with the genetics/cytogenetics of human/mutine hybrid cells, with emphasis on the mapping of human genes by somatic cell hybridization.

The minimum qualification is an honours degree 2(1) in a biological science, although preference will be given to applicants with a Ph.D. or equivalent postgraduate experience. Previous experience of mammalian cell culture would be an advantage, but is not essential. Salary, dependent upon age, qualifications and experience will be on a scale £2,019 to £3,636 or £4,896 with F.S.S.U. benefits.

Applications in writing by December 16, 1974, giving full personal particulars and names of two referees, to the Administrative Officer, MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, (2002)

#### INSTITUT MAX VON LAUE—PAUL LANGEVIN GRENOBLE\_FRANCE

The Institut Max Von Laue—Paul Langevin operates a high flux reactor providing intense beams of neutrons for studies of condensed matter in the fields of physics, chemistry, biology and materials science. Visitors from universities and research centres in the member countries, France, Germany and the U.K., and resident scientists use the high flux beam reactor and the Institut provides scientific and technical support.

Applications are invited for the following post at Grenoble:

#### ENGINEER FOR THE REACTOR

The successful applicant will assist the Head of the Reactor Department on problems of safety and will also provide assistance to all the Section Heads in the Department on studies required to improve the reliability and safety of the reactor. His duties will also include liaison with the French nuclear authorities. Applicants should possess a degree in engineering or equivalent, qualification and have had considerable experience on the operation and safety of nuclear reactors and in writing safety reports. They should also have a working knowledge of French.

Salary will be according to qualifications, experience and responsibilities. Removal expenses will also be paid and assistance given in seeking accom-

Write for application form to: Mr. D. McConville, Science Research Council, c/o British Rail Engineering Ltd., Swindon Works, Swindon, Wiltshire, SN1 5BW, quoting reference and post applied for.

Completed application form should be returned by December 16, 1974. Ref: ILL/04.

#### DEPARTMENT OF PE UNIVERSITY OF A ELECTRONMICROST

Applications are invited for ant Professor in the area of Electronmt. Exceptional candidates in other areas deed state physics will be considered. Thesed state group at the University of Alberts of eight members in a Physics Departm forty-two faculty members.

The effective date of appoint April 1, 1975. The closing date for applic February 1, 1975.
Send vitae list of publication names of

Send vitae, list of publication names of

three referees to:

Dr J. T. Sample
Chairman Chairman
Department of Physics
University of Alberta
Edmonton, Alberta, T60
(X1911)

#### THE UNIVERSIT MANCHESTI DEPARTMENT OF CHILLTH RESEARCH BIOCHE AND RESEARCH HISTOST

RESEARCH HISTUST

Applications are invited fromemists and histologists to join an existing reiplinary research team investigating vuln periods in developing brain, with an empn the long term sequelae of environmentalnees during fetal life and infancy. Applicantial be post-doctoral, with research experiencibe interested in problems with an ultimatelyin relevance. First class facilities and closs departmental collaboration available in new dischool, including, opportunaties for devel new quantitative histological and neurocheprocedures in conjunction with animal beha studies and clinical paediatric research.

Enquiries to: Professor Jopobing, The Medical School, Stopford Build Oxford Road, Manchester M13 9PT. Tel: 06;-241. (2029)

#### SUPRA REGIONAL, AS SERVICE CENTRE FOR STEROIDRMONES, LEEDS

Applications are invited-fe post of

#### SENIOR BIOCHST

in the above Centre. The po available with effect from January I, 1975. Apits should have substantial laboratory experienceferably with steroid hormones and in theid of radio-

Salary: £3,345 to £4,377.

Enquiries should be made rofessor S. R. Stitch, Division of Chemical Pagy, 26-28 Hyde Terrace, Leeds 2.

Application forms obtainablem the Personnel Department, The Generafirmary, Great George Street. Leeds LS1 3EX. (2052)

## **PARASITOLOGIST**

Chesterford Park is the Research and Elop-ment centre for Fisons Agrochemical Ision, the leading U.K. manufacturer of Agricural

A vacancy has arisen in our Biological Spring Department for a Parasitologist to lethe section investigating the anthelmintic anoccidiostat activity of new compounds usinsmall animal techniques.

Applicants, within the age range 22 to 26 ould have a degree in parasitology, or an nours degree in Zoology with specialisation in asitology, and preferably 1 to 3 years postiduate industrial experience. The post would be litable for someone already working in either peside or pharmaceutical resarch, and seeking advarment.

A competitive starting salary will be orted in accordance with career record to date. Or conditions of employment include a first classension scheme, profit sharing bonus, and fou weeks holiday (after 1 year's service). Rocation assistance is available where appropriate.

Research Station, Nr. Saffron Walden, Essex, CB10 1XL.

assistance is available where appropriate.
For an application form, please write, auoting reference number 674/197 to:
R. J. Down, Personnel Officer,
Fisons Limited,
Agrochemical
Division.
Chesterford Park
Research Station

**Pesticide** Research

#### CHNICIAN

A.I.M.N.C. or other qualification

With exin bacteriology, required for laboratory ig in food microbiology and food-poisonihisms. Research projects encouraged. It facilities for further study such as the kamination in bacteriology.

Application from Personnel Officer, Central Public Healistory, Colindale Avenue, London NW9 51 205 7041. (2031)

#### UNITY OF GLASGOW LECTUR IN PHYSIOLOGICAL CHOLOGY

CHOLOGY

Application vited for the post of Lecturer in Physiological plan in the Department of Psychology, Sill be on the Lecturers' scale £2.118 to £4r annum. Placement will be fixed accordiqualifications and experience. F.S.S.U.

The appoint be expected to teach newly instituted undate courses beginning in 1975 and the depails therefore eager to appoint at the earliestle date.

Further par may be had from the Secretary of the Ur Court, (Room 18), University of Glasgow, DQ, with whom applications, (eight copies), the names and addresses of three referees id be lodged on or before January 6, 197

In reply pleate Ref. No. 3586 M

(2032)

#### PORTSMG AND SOUTH EAST HAMPSHHEALTH DISTRICT PATOGY SERVICE

CENTRAL RATORY, SAINT MARY'S HOL, PORTSMOUTH

HOL, PORTSMOUTH

Basic Grade aemist (probationary or postprobationary) rd in the plasma protein laboratory of the Depnt of Chemical Pathology. This
department is d committed with work for the
Southampton Usity Department of Renal Medicine. Experience be available in all aspects of
clinical biocheri and the successful applicant
will be encourate study for a higher degree.
Impending applican obtain further details from
Dr Durant, Torade Biochemist.

Applications ir H. Miller, Senior Pathologist,
Saint Mary's Hal, Milton Road, Portsmouth,
PO3 6AD. Telee: Portsmouth 22331.

(2033)

#### THE PRMACEUTICAL SOCIETY: GREAT BRITAIN

JOURN OF PHARMACY AND ARMACOLOGY

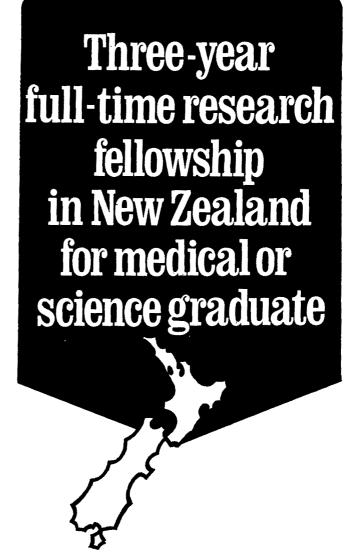
## EDFORIAL **ASISTANT**

The position assistant in the Editorial Department of Journal of Pharmacy and Pharmacology shortly to become vacant. The Journal lishes research papers and reviews on the originating in the disciplines associa with the pharmaceutical and medical spees. The editorial department is unusual being concerned with both editorial and district associations. editorial and pishing procedures.

The person abinted will assist the Editor and will be offd training in all aspects of the duties, whi include the preparation of texts for publicon, library research, proof reading, indexir diagram presentation and liaison with pter and publisher. Salary according to at and experience will be in the range of £538 to £3,024 p.a. (under

Graduates in harmacy or in an allied science who has an interest in the presentation of technal information are invited to apply. Any itential applicant wishing to know more abot the Journal will be a recent copy of request (Telephone 01-405 8967, Ext. 24.)

Details of eduction, qualifications and experience should e sent to the Secretary and Registrar. The Pharmaceutical Society of Great Britain, 17Bloomsbury Square, London WCIA 2NN, in an envelope marked "Appointment J.P.P. (2039)



The Council of the Wellington Medical Research Foundation Inc. invites applications for the whole-time position of Malaghan Research Fellow to be held at Wellington Hospital and/or at Victoria University of Wellington.

The applicant should be a research worker with evidence of

capacity to undertake original research. He must possess a higher qualification such as Ph.D. or Membership of a Royal College of Physicians, or Pathologists. He should indicate an intended research project on which he has embarked or is dedicated to pursue. Whilst the Council will accord preference to an applicant whose project is in the field of Haematology, projects in other fields of medical research pertaining to diseases of the blood will be considered.

The yearly rate of salary shall be as agreed upon between the selected applicant and the Council of the Foundation. It will be within the scale for Specialist Medical Officers, which is \$12,639-\$16,926,\* or for Science Graduates with Ph.D., \$7,601-\$11,774.\* Adjustments will also be made to cover increases in the cost of living in line with Wage Adjustment Orders covering Hospital or University staff.

The appointment is for three years. Assistance towards transport expenses is available.

Conditions of Appointment, method of application and other information may be obtained from the Secretary of the Foundation in New Zealand, or from the Office of the High Commissioner, New Zealand House, London.

Applications close with the Secretary of the Foundation on 28th February, 1975. \*(N.Z. Dollars)

## **WELLINGTON MEDICAL RESEARCH FOUNDATION INC.**

P.O. BOX 3025 WELLINGTON NEW ZEALAND

## PRINCIPAL PHYSICIST

Required for

#### KING'S HEALTH DISTRICT

and

#### S.E. THAMES REGIONAL HEALTH AUTHORITY

Applications are invited for the above appointment in the Department of Medical Physics, King's College Hospital, with some responsibilities in other hospitals of the Region. The successful applicant will have had wide experience in medical physics, and will be responsible to the Director of the Department for such work of the Department involving ionizing radiation as may be agreed. This work may include physics in Radiotherapy, Nuclear Medicine and Radiation Protection at King's College Hospital and Medical School, Nuclear Medicine Physics at the Brook Hospital, Woolwich, and Radiation Protection in more than 60 hospitals in the S.E. Thames Region.

The Department of Medical Physics is attached to King's College Hospital Medical School and University of London, and the successful applicant will take part in the research and teaching activities of the Department.

Further information and application forms from the Personnel Office, King's College Hospital, Denmark Hill, London SE5 9RS.

Tel: 01-274 6222 Ext: 2724/8.

Closing date December 16, 1974.

(2037)

#### UNIVERSITY OF ZAMBIA

Applications are invited for the following posts in the School of Medicine:-

1. PROFESSOR AND SENIOR LECTURER AND LECTURER IN DEPARTMENT OF SURGERY.

OF SURGERY.

Candidates must possess a recognised postgraduate qualification in Surgery. Professorial candidates must have distinguished themselves in a field of Surgery and have several years experience of teaching and administration in a University. A detailed list of scientific publications is required from all candidates as well as a full statement of educational and professional training and positions held. Appointee to Professorial post will be expected to head the Department of Surgery while appointees to the Senior Lectureship will act as Consultant and Senior Registrar, respectively.

2. PROFESSOR/SENIOR LECTURER IN DEPARTMENT OF PATHOLOGY AND MICROBIOLOGY.

Candidates should have specialised in either Basic Immunology or Clinical Immunology or Immunopathology. They should possess a medical degree and an appropriate postgraduate qualification or M.R.C. Path (or equivalent), and adequate experience in the field of Immunology. Non-medically qualified candidates whose basic training is immunology will also be considered.

- 3. SENIOR LECTURER/LECTURER IN BIOCHEMISTRY in Department of Physiological Sciences. Candidates must possess a posterior data and the control of the control logical Sciences. Candidates must possess a postgraduate qualification in Biochemistry and for Senior Lectureship level, teaching experience and a list of scientific publications are required. Appointee will be required to teach third year medical undergraduates and conduct research.
- 4. SENIOR LECTURER/LECTURER IN ANATOMY in Department of Anatomy. Candidates should be graduates with postgraduate qualifications in Anatomy. Preference will be given to those with five or more years experience in undergraduate Anatomy teaching and with interest in the research fields of Cytology and Histochemistry. Appointee will be responsible to the Head of the Department and will be expected to participate in Histology and Medical Embryology teaching of 3rd year medical students.

SENIOR LECTURER/LECTURER IN DEPARTMENT OF CHEMICAL PATHOLOGY.

5. SENIOR LECTURER/LECTURER IN DEPARTMENT OF CHEMICAL PATHOLOGY.

Candidates should be medically qualified and must possess M.R.C. Path or equivalent with relevant teaching and research experience. Non-medically qualified candidates who have experience as Clinical Biochemists will also be considered. Appointee will be expected to teach undergraduate and postgfiraduate medical students, conduct specialist services for the University Teaching Hospital staff and take part in research.

Salary scales: Professor K7,400 to K7,800 p.a. Senior Lecturer K5,600 to K6,600 p.a. Lecturer K4,000 to K5,400 p.a. Medical differential allowances may be payable locally. (£1 sterling=K1.50). The British Government may supplement salaries of married Professors, Senior Lecturers and Lecturers in range £516 to £1,152 p.a. (sterling) or salaries of single Professors and Senior Lecturers in range £78 to £204 p.a. (sterling) (normally free of all tax). This supplementation is unlikely to be applied to single appointees to Lecturer level. These rates are for academic staff, other than clinical medical staff. The rates for clinical medical staff are currently under review. There may also be provision of children's educational allowances and holiday visit passages, family passages; various allowances; superannuation and medical aid schemes; regular overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, not later than December 30, 1974 to the Registrar, University of Zambia, P.O. Box 2379, Lusaka, Zambia, Applicants resident in UK should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London WIP 0DT. Further particulars may be obtained from either address. (2056)

#### POSITIONS IN GEORY

Scarborough College, Universitanto, expects to make 3 appointments inhy effective July 1, 1975, subject to tolity of funds. Applications in any field ansidered but preference will be given dates with research and teaching interests in:

(i) Economic Geography
(ii) Hydrology, water resoul
(iii) Urban Systems
Applicants with a strong teaterest in

Applicants with a strong teaterest in quantitative analysis and/or rensing/air photo analysis would be welcom-trgraduate teaching responsibilities are at Sch College with opportunity for graduate ten the St. George Campus, University of Appointment may be made at any rankssistant to full professor and may be on a or permanent basis. Salary is open. Candould have a Ph.D. or be in the final stagt.D. work. Letters of application with cur vitae and the names of three referees sh submitted to:

Professor Brian Greenwood
Assistant Chairman
Division of Social Sciences
Scarborough Coffese
University of Toronto
West Hill, Ontario
Canada MIC 1A4

(2038)

## **MEDICA** RESEARCH CNCIL

## Neuropsychry Unit

Research Assistant i Junior Technical Officer gradenired for medical research involvbiochemical studies on the n. B.Sc. preferred. Salary-accordo age and qualifications. Apply to 3. Balazs, Medical Research Co., Neuropsychiatry Unit, **W**mansterne Road, Carshalton, Surre (2036)

#### UNIVERSITY OF CBRIDGE CAVENDISH LABOORY

CAVENDISH LABOORY

The High Energy Physics Reh Group of the Cavendish Laboratory wishes recruit suitably qualified scientist or engineer t responsible for the hardware aspects of an matic computer-controlled particle track measusystem used for the analysis of elementary par experiments by the bubble chamber and relateethods. The system is based upon a high pren programmable laser-beam scanner and digitizdich the successful applicant will be required tlapt and develop for future experiments undertal by the Research Group. The applicant should i considerable experience of the electronics de, instrumentation and systems aspects of comptontrolled equipment. Some experience of menical and optical design is desirable. Familiarity a the high energy physics field and possession of a.D. degree would be advantageous. be advantageous.

The post carries a starting ary in the range £2,580 to £4,896 p.a., the entroint depending on age and qualifications. F.S.5 membership is available. Applications, includinames of referes, should be addressed to: The retary, Cavendish Laboratory, Madingley Road Cambridge, CB3 OHE. (2043)

#### UNIVERSITY OF LEADING RESEARCH ASSTANT

RESEARCH ASSIANT
required in Department of Soil Science from February 1, 1975 to study t) pore structure of soils in relation to propertit in the field. The person appointed will develop techniques and use existing mercury intrusion ad similar methods. Salary approximately £2,200 p. according to qualications and experience. App, quoting Ref. M. 56, to Assistant Bursar (Personel), University of Reading, Whiteknights, Reading RG6 2AH.

#### UNIVERSITY OF BATH

Applications are invited for the post of PROFESSOR OF BIOCHEMISTRY

from persons experienced in any area of biochemistry. Further particulars obtainable from Senior Staff Officer, The University, Bath BA2 7AY. (2051)

#### LEEDS AREA HEALTH AUTHORITY (TEACHING) WESTERN DISTRICT SUPRA-REGIONAL LABORATORY

FOR LEAD ASSAY, LEEDS Applications are invited for a new post of post-probationary grade

#### BIOCHEMIST

for this laboratory, which will be operated by the Department of Chemical Pathology, University of Leeds. Applicants should have experience in clinical chemistry, a knowledge of instrumentation and, if possible, trace metal analysis. The successful candidate will be in day to day charge of the laboratory and will be given opportunities for further training and participating in the activities of the Department.

Enquiries can be made to Mr. P. M. G. Brough.

ment.

The properties of the made to Mr P. M. G. Broughton, Department of Chemical Pathology, Géneral Infirmary, Leeds.

Applications, giving the names of two referees, should be sent within 14 days of the appearance of this advertisement to the Personnel Officer, The General Infirmary, Great George Street, Leeds LSI 3EX. (2053)

#### ROYAL POSTGRADUATE MEDICAL SCHOOL TECHNICAL OFFICER

required in Endocrine Unit to assist in clinical studies on disorders of calcium and bone metabolism. Salary on scale £2,268 to £3,729 per annum plus threshold allowance, according to age and

experience.

Applications to the Personnel Officer, (743 2030 Ext. 93), R.P.M.S. Hammersmith Hospital, DuCane Road, London W12 0HS, quoting ref. no. 2/489.

(2066)

## **Oligotrophic Lakes Research Scientist**

Canadian \$15,800 to \$25,600

The ONTARIO MINISTRY OF NATURAL RESOURCES, Fish and Wildlife Research Branch, requires an oligotrophic lakes research scientist to devise, direct and participate actively in research and experimental management to develop and apply effective methods of assessing stocks and allocating surplus yields in the multi-gear, multi-species, multi-agency fisheries of Lake Superior and related waters; contribute new understanding of aquatic production systems by participating in multi-disciplinary research in systems ecology. Location: Maple, Ontario, Canada (approximately 20 miles from Toronto.)

Qualifications: Ph.D with solid training in applied mathematics and statistics, biometrics and systems ecology; experienced in the use of computers as well as in biology preferable; specialization in fisheries preferable, but not necessary; extensive experience in field and laboratory work, preferably in multidisciplinary research teams; published contributions earning international recognition.

Please submit detailed resumes by December 31, 1974, to:

Recruitment Officer, Resources Branch, File NR412, Civil Service Commission, Parliament Buildings, Toronto, Ontario, M7A 1Z5, Canada.

This position is open equally to men and women.



**Ontario** Public Service (2041)

## **ENTOMOLOGIST**

Chesterford Park is the Research and Development centre for Fisons Agrochemical Division, responsible for the invention, selection and development of novel chemical compounds for the protection of crops, public health and materials. Internal transfers have created two immediate vacancies for Entomologists in our Zoology Department.

Entomologist

The duties of this post will be to conduct and evaluate both laboratory and field trials of candidate new pesticides (insecticides, acaricides and nematiwhich may overseas duty for periods of up to six months in any one year. After suitable training, full responsibility will be given for specific departmental projects. Applicants will probably be in the age range 24 to 28, with a good honours degree in Zoology/Entomology or Applied Biology, and have some post graduate experience of Applied Entomology and a sound know-Entomology and a sound know ledge of laboratory and field working experimentation. knowledge of French, German or Italian would be advantageous, (Ref. No. 671/197).

Senior Entomologist

The successful applicant would be closely involved with the planning and execution of applicant the annual trials programme of candidate new pesticides, for which it is envisaged he would

ultimately assume total responsibility. The preferred upper age limit is 35 and candidates should possess a good honours degree in Entomology (Ph.D. is not essential) with several years post graduate experience of Applied Entomology, a thorough knowledge of laboratory and field techniques of pesticide evaluation and preferably a specialised knowledge of these. Considerable travel, both within the U.K. and overseas is likely to be involved. (Ref. No 674/197).

Starting salaries will be competitive and in accordance with career record to date. Other company conditions of employment include a first class pension scheme, a generous sick pay scheme, profit sharing bonus and four weeks leave (after one year's service). Assistance with relocation expenses will be available where appro-

For an application form for either post, please write, quoting the appropriate reference to: Roger Down, Personnel Officer, Fisons Limited Agrochemical Division, Chesterford Park Research Station, Nr. ford Park Research C.S. Saffron Walden, Essex, CB (2048) ĊB10

Pestidde Research



## UNIVERSITY OF DUBLIN Trinity College

## **CHAIR OF MICROBIOLOGY**

Applications are invited for appointment to this Chair, which will fall vacant on July 1, 1975.

Further particulars may be obtained from:

The Secretary to the College West Theatre Trinity College Dublin 2.

Formal applications should, if possible, reach the Secretary before January 6, 1975. (2061)

# Opportunity Oversea

## Indonesia

## **Weed Science** Consultant

To assist in the supervision of trainees and the organisation and lecturing involved in short term training courses; advise on the current and future programme of the project and the development of equipment and facilities; conduct a research project relevant to the BIOTROP programme.

Candidates should possess at least an M.Sc. in Weed Science or education allowances and free accommodation and medical Some teaching experience would be an advantage. Appointment 2 years. Salary in scale £4,500 to £6,500 p.a. plus a tax free allowance in scale £620 to £1,255 p.a.

Other benefits include paid leave, free family passages, children's closely related subject with experience in tropical weed research. attention. Applicants should normally be citizens of and permanently resident in the United Kingdom.

For full details and an application form please apply giving age and brief details of qualifications and experience to:-Appointments Officer

Ministry of •

Overseas Development

Room E301 Eland House Stag Place London SWIE 5DH

(2046)

#### ARTIFICIAL KIDNEY TECHNICIAN

required at The London Hospital (Whitechapel) for maintenance of the artificial kidney machines, both at the

artineral kidney, machines, both at the hospital and in patients homes.

Experience in Dialysis Unit an advantage but not essential.

Training will be given but candidates should have had experience in electronic and mechanical engineering and may have O.N.C. or a higher qualification to support them. Driving licence required. licence required.

Salary according to qualifications and experience. Further details from Alan Hawkes (Chief Technician) 01-247 5454 Ext. 286.

Applications to: Moira Payne, dministrator, Personnel Services, Administrator, Personnel Services, The London Hospital (Whitechapel), London El 1BB. Tel: 01-247 5454 (2042)Ext. 388.

#### UNIVERSITY OF ZAMBIA

Applications are invited for the following posts in the School of Medicine:

CHIEF TECHNICIAN/SENIOR TECHNICIAN with experience in HAEMATOLOGY, BIOCHEMISTRY or MICROBIOLOGY. Candidates should hold F.I.M.L.T. or equivalent, Appointee will be responsible for the preparation of materials and the daily running of teaching laboratories.

the daily running of teaching laboratories.

OHIEF TECHNICIAN/SENIOR TECHNICIAN in sub-Department of HAEMATOLOGY and CHIEF TECHNICIAN/SENIOR TECHNICIAN in sub-Department of PARASITOLOGY Applicants should hold F.I.M.L.T. and be experienced techicians. Appointees will prepare and supervise practical classes for medical students and trainee technologists; assist with research to the respective sub-Departments and become involved in specialised investigations for the University Teaching Hospital.

investigations for the University Teaching Hospital.

Salary scales: Chief Technician K3,300 to K4,900 p.a. Scnior Technician K2,900 b. K3,500 p.a. (£1 sterling = K1.50). The British Government may supplement salaries of married appointees in range 2456 to £516 p.a. (Sterling) (namally free of all tax). This supplementation is unlikely to be applied to single appointees. There may also be provision of children's education allowances and holiday visit passages. Family passages; urious allowances; superannuation and medical ad schemes; regular overseas leave. Detailed applications (2 copies), including curriculum vitae and taming 3 referees, should be sent by airmail, not ler than December 30, 1974 to the Registrar, University of Zambia, P.O. Box 2379, Lusaka, Zamta. Applicants resident in U.K. should also sent I copy to Infter-University Council, 90/91 Tottaham Court Road, London, WIP ODT. Further particulars may be obtained from either address. (2055)

UNIVERSITY OF JUNDEE DEPARTMENT OF PHAMACOLOGY AND THERAPETICS

#### RESEARCH ASSISANTSHIP

Applications are invited from University graduates or holders of edvalent qualifications for a Research Assistaship in the Department of Pharmacology hd Therapeutics, located in the new Ninewis Hospital and Medical School complex, Didee. The holder of this post, which is avaible immediately will be required to prove a Gas-liquid Chromatographic Service whin the Department and to collaborate as quired with other staff in research and teacing and will be afforded facilities to carry or his own research work on a part-time basis for a higher degree. Applicants should ideallysave had experience of gas-liquid chromatoraphy but training in this will be available

The salary attached to he post will be within the range £1,311 to£2,052 depending on qualifications and experience. Applicants should apply, living their qualifications, the names of two eferces and quoting reference Est/39/741 y December 20, 1974 to: The Secretary The University, Dundee DD1 4HN, from hom further particulars may be obtained. (2044)

#### ARIE CURIE MEN FOUNDATION THE, OXTED, RHS OTL.

1. RESEARISTANT for the Biological Chemistry UrRAD.R.I.C. or B.Sc. (Hons) in chemistry, interest in organic chemistry. Current project the design, synthesis and evaluation of tumour agents and metabolism of anti-dugs.

2. RESEARISTANT for the Metabolic Unit with a n Nutrition or Biochemistry and an interchical research in cancer. Experience of dbolism would be an advantage.

tage.

3. TECHNIce Cell Biology Unit requires a technician keen interest in original research, to well surface changes associated with malignan Salaries are n the Whitley Council Scale with superannulesearch Assistants may register for a highe after an initial probationary period. Applicacluding the names of two referees and it the post sought should be sent to the Scat the above address.

(2060)

#### BIEK COLLEGE (UNITY OF LONDON) DEPARTMF CRYSTALLOGRAPHY RECH OFFICER

RECH OFFICER

required to wo: a team concerned with the structure and r water in biological systems. The Research Gyill concentrate on biological macromolecules, there will be considerable scope to develcher own ideas. A chemical, biochemical, ortal background is necessary, and some crystabic experience of proteins is desirable, thoug essential.

Starting salarya range £2,118 to £2,757 plus London Allowal £213. Maximum period of appointment thris from February 1, 1975, or as soon as possereafter.

Application found further details from Dr John Finney (01622 Ext. 420).

#### VICTORUNIVERSITY OF VLINGTON ( ZEALAND

#### CHAIR OFRE MATHEMATICS

Applications afted for the above-mentioned Chair from persotably qualified in any branch of the subject essor Reuben Sandler, who currently holds thair, is resigning to take up activities of a dnt nature. The Department would welcome inal enquiries from interested persons.

persons.

Salary: NZ\$1 to NZ\$19,233 p.a. An allowance towardavel and removal expenses within specified k.

Further particle and application procedure obtainable from tlssociation of Commonwealth Universities (App 36 Gordon Square, London WC1H OPF.

Applications cleebruary 15, 1975. (2074)

## SENIORTECHNICIAN

(IMUNOLOGY)

This new d expanding Institute requires a Sor Technician experienced in thelld of immunology to join a team vking on the standard-isation of algens. Techniques em-ployed inclu isoelectric focussing and radioimnoassays plus many other immungical skills. The post offers a great al of interest and provides a good portunity for a Technician of seprity holding at least HNC, HND requivalent to widen his or her eperience in a rapidly developing 60 developing fie

Salary: Seor Technician Scale £2,646 to £3,6) per annum including London Weitting (under review) plus Threshol Payments.

Applicants hould write quoting ref. No. 0037 b R. S. Dunn, Personnel Officer, Naional Institute for Biological Standards & Control, Holly Hill, Hampsted, London NW3 6RB or telephone 11-435 2232 Extn. 16.

An application form will be sent to (2040)

#### UNIVERSITY OF QUEENSLAND AUSTRALIA

#### LECTURER IN ORAL BIOLOGY DEPARTMENT OF DENTISTRY

Applicants should have appropriate qualifications and research interests in any of the following fields: biochemistry, histology, microbiology or pathology. Oral Biology deals with the study of oral tissues in health and disease in Queensland, the sub-department also teaches general histology and general pathology to dental students.

Salary within the range \$A9,002 to \$A12,352 plus \$A678 (Clinical Loading) per annum.

Other Benefits: Superannuation similar to F.S.S.U., housing assistance, study leave, travelling and removal expenses.

Additional information and application forms are obtainable from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H OPF.

Applications close on January 17, 1975.

#### UNIVERSITY COLLEGE OF NORTH WALES BANGOR

DEPARTMENT OF ZOOLOGY

Applications are invited for the post of LECTURER IN ZOOLOGY

The appointment will take effect not later than October 1, 1975. Applicants should have an interest in either ecology or animal behaviour.

Salary will be on the scale £2,118 to £4,896. according to age, qualifications and experience.

Further particulars may be obtained from the Secretary and Registrar and applications (two copies), giving details of age, qualifications and experience, together with the names and addresses of three referees, should be sent to reach the Secretary and Registrar, University College of North Wales, Bangor LL57 2DG, by Friday, January 17, 1975. (2080)

#### Ministry of Agriculture, Fisheries and Food Food Science Division, London

## **Chemist/Biochemist**

■ In Food Chemistry, Composition and Safety Branch ■ Obtain, summarize and evaluate scientific information on manufacture and composition of food, including additives and contaminants 
Develop and maintain broad interest in food processing, composition and legislation Prepare critical review papers and participate in committee work.

☐ 1st/2nd hons degree in Chemistry, Biochemistry or Food Science ☐ Age under 27 ☐ Appointment as Scientific Officer (over £2150 to around £3250) ☐ Ref: SB/16/AE.

## **Chemist/Physicist**

■ In Food Technology Section ■ Join team studying packaging, storage and warehousing practices ■ Assist in practical laboratory and field trials . Some inspection and investigational work involving travel in U.K.

Degree, HNC or equivalent in Chemistry, Physics or Food Science Current driving licence an advantage  $\Box$  Age under 27  $\Box$  Appointment as Scientific Officer (over £2150 to around £3250)  $\Box$  Ref: SB/17/AE.

#### Ministry of Agriculture, Fisheries and Food Food Science Division, Norwich

■ To take charge of three projects concerned with reactions of additives and contaminants in food ■ Programme relates to food safety and includes work on sulphites, nitrites and packaging constituents Relate chemical changes to conditions experienced in processing, storage and cooking.

☐ Good hons degree or equivalent in Chemistry or related subject ☐ 4 years' post-graduate research experience, including analytical methods for trace compounds in complex media [] Organic chemistry bias [] Age under 32 [] Appointment as Senior Scientific Officer (over £3300 to around £4600) 
Ref: SB/15/AE.

Application forms (for return by 19 December 1974), from Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 IJB, telephone Basingstoke 29222 ext. 500 (or, for 24 hour answering service, London 01-839 1992).



# Graduate

Shell Research Limited, at their Sittingbourne Research Centre in Kent, have a vacancy for a graduate chemist, specialising in organic mass spectrometry, to work in the Physics Division of the Milstead Laboratory of Chemical Enzymology. You should have a PhD and preferably some post doctoral experience, under a recognised authority, in the field of mass spectrometry. You will join a team providing an analytical service to the Sittingbourne Research Centre and act as a consultant to other locations on site which operate their own glc-mass spectrometers. Full support services are available but there will be some opportunity to carry out independant research which will actively be encouraged. The job offers exceptional facilities for research in the field of mass spectrometry. At present the instruments available consist of a MS30 double beam, double focusing mass spectrometer coupled to a gas chromatograph; a MS902 double focusing mass spectrometer which currently is being equipped with facilities for chemical ionisation and field ionisation/field desorption; and a VG2040 data handling system. The level of appointment will depend on background and experience and the salary offered will be competitive. Please write giving details regarding personal background and experience or write/telephone for an application form to: Shell Research Limited, Recruitment Division (N), PNEL/34, Shell Centre, London SE17NA. Telephone 01-934 2948.



(2057)

NATIONAL INSTITUTE FOR MEDICAL RESEARCH MILL HILL - LONDON

## JUNIOR TECHNICAL OFFICER

required in the Division of Parasitology to assist in research on antigens of malaria parasites. Suitable for graduate in biochemistry. Salary on scale £2,104 to £2,947 p.a. Please apply, quoting ref: JTO/PARA to Mrs M. Y. Jim, Assistant Personnel Officer, N.I.M.R., The Ridgeway, Mill Hill NW7 1AA. Telephone: 01-959 3666.

(2084)

## **Micropalaeontets** and Palynole

Positions exist in Robertson International's Laboratories in Wales, Singapore and Calgary Aphould have experience in Tertiary/Microfaunas or Microfloras.

Robertson Research Internal an independent consultancy organiccialising in a large Jvariety of to the petroleum and mining industrently over thirty resident micropalasts and palynologists are employed iroleum division worldwide.

All posts are permanent andable.

Please apply to the Personer: Robertson Research Intern Limited, Ty'n-y-Coed, Llanrhos, Llandudno, LL30 ISA, Gwynedd, North Wales, U.

#### UNIVERSITY OF WITWATERSR JOHANNESBURG, SOURICA VACANCY:

SENIOR LECTURER IN PA WITH SPECIAL RESPONSIBILIN THE FACULTY OF EDUN

Applications are invited for over post it the Department of Physics.

The principal duty attached to organise in-service trainin physical science in high school. The salary scale attached to st is R8.4 by R360 to R9.900 by R450R11,250 (£1 R1.62 approx.). The initial salarbe determin according to qualifications and ence.

Benefits include an annual pension a medical aid, and a housing su(if eligible). Intending applicants should, the information-sheet relating to this post the Registr University, of the Witwater, Jan Sm Avenue, Johannesburg, South a, with whapplications should be lodget, later the December 15, 1974, U.K. apis, may obt the 'information sheet' relating his post from the London Representative, ersity of Witwatersrand, 278 High-Holboondon, W.C.

#### UNIVERSITY OF ITERN AUSTRALI PERTH PHYSICS!

Applications for appointment wo position: LECTURE

in the Department of Physice invited for persons with interest and rience in teaching of physics and withearch interpretably in one of the wing fields: Experimental: gravity wave deen, low tenture or solid state physics; (b) oretical: gerelativity, plasma physics, (tum mechastatistical mechanics or thermiamics.

The appointments will be nne year inite the possibility of exten for a signar or permanency. The cun salary rang a Lecturer is: \$A9,002 to 112,352 p.a. appointee from within Austrawould be ento fares to Perth for self wife and elsewhere to appointment enses of \$A1,400. Further informationary be obfrom the Staffing Officer.

Applications in duplicate ling full perarticulars, qualifications, pications, te and research experience an earliest davailability (preferably not laithan April should reach the Staffing Cer, Univerself Western Australia, Nedlands/Vestern Australia, Nedlands

Biology Department Chairrson for un graduate and graduate deptement. Requ capabilities: leadership teaching research programs, execute skill.

Address letter of inquir and resum Dean Warren Ilchman, ollege of Li Arts, Boston University, 75 Commonw Avenue, Boston, MA 0225.

An Affirmative Action/qual Oppor Employer. (20

### PHMACIST :

require search and development tories. Applicants must hicted Pharmaceutics as theirlity, with some experienceearch and development. Stommensurate with age andrience. Application forms fr

> Thestary Biomoratories Ltd. Bionuse. Cany Villas, Lonel 2HB.

> > (2059)

### LA T. UNIVERSITY MELNE, AUSTRALIA

READER I'RE MATHEMATICS

Applications vited from suitably qualified candidates for sition as Reader in Pure Mathematics. accessful applicant will be expected to m major contribution to the teaching and the of this rapidly growing Department.

Salary: SA16p.a. F.S.S.U. type super-annuation,

Further inform and application forms are available from ssociation of Commonwealth Universities (Ar 36 Gordon Square, London WCH Opf om the Registrar, La Trobe University, Bune Victoria, Australia, 3083.

Application. 11 1978. Applications on January 31, 1975.

### QUEEMRY COLLEGE UNIVITY OF LONDON DEPARNT OF PHYSICS

Applications invited for a postdoctoral RESEAR ASSOCIATESHIP

to study crystal cture, phase transition and thermal motion iolecular crystals using low-temperature X-raid neutron diffraction techniques. The researcy of the A.E.R.E., well and possibly also at the I.L.L., Grenoble.

Initial salary £8 to £2,247 per annum and £213 London Allace. Applications in writing (including currien vitae and names and addresses of two rees) should be sent as soon as possible to TRegistrar (N), Queen Mary College, Mile Entoad, London E1 4NS.

(2081)

### UNERSITY OF NEWCASE UPON TYNE ORGANIC OCHEMISTRY UNIT Applications invited for the post of SENIOR REARCH ASSOCIATE (£2,0 to £3,285)

### RESEARH ASSOCIATE (£2, 8 to £2,412)

in the ORGANIC EOCHEMISTRY UNIT for research within theireld of organic geochemistry and petrology of ecent and fossil sediments, including coals.

including coals.

The Unit is with the Department of Geology and the post would e suitable for candidates with a background of gelogy or chemistry or preferably both. In the first instance the post is available until Aril 30, 1976, commencing January 1, 1975, is by arrangement. Starting salary will be accoding to age and experience. Applications including a brief curriculum vitae and the names of two referees should be sent as soon as possible to: Dr. D. G. Murchison, Organic Geochemistry Unit, The University, Porter Building, St. Thomas St. Newcastle upon Tyne NEI 7RU.

# Microbiologist/ Fermentation Technologist

The R & D Division of G. D. Searle & Co. Ltd., a worldwide ethical pharmaceutical organisation, has an opening for a graduate to join a department working on the growth and physiology of tissue culture cells. The candidate would initiate research into novel methods of growing cultured mammalian cells and would develop this work to pilot plant scale. Excellent laboratory and pilot plant facilities are available for this project.

The post would ideally suit a good honours graduate in microbiology or related subject with an interest in fermentation technology. Previous experience is desirable but not essential.

An attractive commencing salary will be offered and conditions of employment include 4 weeks' holiday, Pension Fund Scheme, BUPA Scheme, Staff Restaurant and an active recreation club. Assistance with relocation expenses will be given in appropriate circumstances. Initially, please contact: H. W. Čooke,

Personnel Manager, R & D Division, G. D. Searle & Co. Ltd., Lane End Rd., High Wycombe, Bucks. Telephone: High Wycombe 21124 Ext. 3283. Please quote Reference: RA/118.



(2079)



## Administrative Assistant

We have recently appointed a Project Manager to be responsible for many of the long-term toxicological studies carried out in the Laboratory. He needs a graduate assistant with biological knowledge and secretarial training. The assistant's main duties would be:assisting with long-term planning, arranging and minuting project meetings, ensuring that relevant toxicological information is available to project teams, acting as a communication link between the Project Manager, scientists and ICI Divisions, collecting, assembling, sub-editing and issuing toxicological reports and acting as a personal secretary to the Project Manager.

This will be an interesting and challenging appointment in a newly-formed Group.

Applications and enquiries should be addressed to:-

Miss A. Waring, Personnel Officer, Imperial Chemical Industries Limited, Central Toxicology Laboratory, Alderley Park, Nr. Macclesfield, Cheshire SK10 4TJ.

Tel: Alderley Edge 2711 Ext 156.

(2086)

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD Fisheries Laboratory Burnham-on-Crouch

# RINE ECOLOGIST

HIGHER SCIENTIFIC OFFICER to carry out fundamental research on bethos/sediment relationships in support of a team carrying out routine ecological investigations related to pollution around the coast of England and Wales. The study will involve field work in coastal situations and trips to sea in research vessels.

The appointment will be for period of three years during which the applicant will be required to complete a specific programme of investigations under the general direction of the project leader.

QUALIFICATIONS: A 1st or 2nd class honours degree and have at least 3 years postgraduate research experience preferably benthic ecology or in a related subject. Ability to work at sea essential.

AGE: Under 30.

SALARY: £2,461 by 8 increments to £3,371 p.a., also threshold increases. Short-term gratuity.

APPLICATION FORMS are available from Mr S. A. COLLINS, Ministry of Agriculture, Fisheries and Food, Room 504, Victory House, 30-34 Kingsway, London WC2B 6TU. Telephone No. 01-405 4310 Extn 20 and 21.

Closing date for completed applications: December 20, 1974.



# **Biochemists**/ **Chemists**

Applications are invited from graduates in biochemistry or chemistry to fill two vacancies at the Laboratory. The successful applicants will be required to assist in solving problems concerning the biochemistry and physiology of (a) pesticides and herbicides and of (b) novel food supplements in mammals.

We would like to appoint people with 2 or 3 years experience in such work but would consider applications from recent graduates.

Applications and enquiries to:-Miss A. Waring, Personnel Officer, Imperial Chemical Industries Limited, Central Toxicology Laboratory, Alderley Park, Nr. Macclesfield, Cheshire SK10 4TJ.

Tel: Alderley Edge 2711 Ext 156.

(2083)

### UNIVERSITY OF ST. AL DEPARTMENT OF CHE ION MOLECULE REA

Applications are invited to of S.R.C. Postdoctoral Research to carry out research in the field decule Reactions. Applicants must pience in mass spectrometry or rea of chemistry or chemical physicalary will be in the range £2,118 2 per annum, plus F.S.S.U. Application with the names of two refeld be sent to Professor J. M. Tedde Building, The University, St. Andre Ky16 9ST on or before January 1, 1058)

### M.R.C. LABORATO MOLECULAR BIO JUNIOR TECHNICALDER

required to join an organic fry group working mainly on peptide syl-andidates should possess a degree, H.N. equivalent qualifications. The successful aprill assume full responsibility for a small scopic and analytical laboratory and will ate in the experimental research of the gaittal salary in the range of £1,626 to with good prospects for promotion and picy.

Applications giving details alifications, experience, and the names of trees should be sent to Dr R. C. Sheppard, Laboratory of Molecular Biology, Hills Rembridge, as soon as possible.

Pleas mentii this journl whei answerng

advertisenents



### **Pharmacologist**

Reroducts Limited is the United Kingdom subsidiary oge and successful pharmaceutical company, with a law Research Laboratory in our Welwyn Garden City, Hishire, site.

Wseeking a graduate with relevant experience to join a working in the field of neuropharmacology. The perpointed should have a B.Sc. in physiology or phology and since graduation should have become falwith electro-physiological techniques.

Ouratories provide facilities and working conditions of a vgh standard; in addition we can offer Conditions of Sewhich are attractive, including generous assistance wification expenses in appropriate cases.

Apions for this post quoting reference RD should be add to the Welwyn Personnel Manager. Closing date Deer 20, 1974.



Roche Products Limited elwyn Garden City, Hertfordshire AL7 3AY

FELLOWSHIPS AND STUDENTSHIPS

### TE UNIVERSITY OF MANCHESTER **IPARTMENT OF CHILD HEALTH**

### CINICAL RESEARCH **FELLOWSHIPS**

The optimity arises of awarding two Clinical Research Fellowships to join a fulime research team within the Department of Child Health. The main, but of exclusive interest of the team has been the development of the brain id its vulnerability, with an emphasis on the long term sequelae of environmental influences during fetal life and infancy. The present aim is that climans preparing for a career in a University Clinical Department should he to extend the work of the present team into the clinical area; and this dild be in Paediatrics, Obstetrics, Developmental Behavioural Sciences, evelopmental Pathology, Epidemiology, or any other field of clinical releance to the team's central interest. Appointments are likely to be at the ost-M.R.C.P., Registrar/Senior Registrar level, and should lead to a highe Degree. Enquiries to Professor John Dobbing, The Medical School, Stoford Building, Oxford Road, Manchester M13 9PT. Tel: 061-

### LIVERPOOL POLYTECHNIC SCHOOL OF PHARMACY SRC STUDENTSHIP IN MEDICINAL CHEMISTRY

Applications are invited from candidates with a good honours degree in Pharmacy or Chemistry, or Grad. R.I.C. to work for a Ph.D. degree on the project "4-phenylpiperidine derivatives as potential non-addicting analgesics". The work will involve organic synthesis and stereochemical Audies

The Project is a C.A.S.E. award and the co-operating body is Allen and Hanburys Ltd. The S.R.C. stipend applies and this may be supplemented by payment for teaching duties and income during the industrial period.

Enquiries and applications to A. F. Casy, D.Sc., F.P.S., School of Pharmacy, Liverpool Polysechnic Byrom Street, Liverpool L3 3AF, Tel: 051-207 3581.

### BEIT MEMORIAL FELLOWSHIPS FOR MEDICAL RESEARCH 1975

Notice is hereby given that an election Junior Fellows to begin work on October 1, 1975 will take place in May 1975. Junior Fellowships have annual values within the range of £2,000 to £2,525 p.a. (plus London Allowance £213 p.a.) plus yearly increments of £175 for 3 years. As a superannuation benefits are provided for which the successful candidate will be required to contribute 61% of the animal stipend and to which the Trust will make a contribution of 12% Fellows who are already members of the Federated Superannuation System for University or of the National Health Services Superannuation Scheme may opt to remain in their existing scheme, which case the contributions will be at the rates appropriate to them. Candidates must have taken a degree in a faculty of a university approved by the Trustees in Her Majesty's Dominions, Protectorates and Mandated Territories, India, Pakistan, the Republic of Ireland and the United Kingdom, or a medical diploma registrable in the U.K. Elections to Junior Fellowships are rarely made above the age of 35 years. Applications from candidates must be received not later than March 21, 1975. Candidates must submit evidence that they can be given accommodation in the departments where they propose to work, which must be in Great Britain or Ireland.

Forms of application and all information may be obtained from Professor W. G. Spector, Pathology Department, St. Bartholomew's Hospital, London ECIA 7BE. For overseas candidates forms of application may be obtained from The Secretary, South African Medical Council, P.O. Box 203, Pretoria, South Africa; The Ministry of Health, The Government of India, New Delhi, India; The Secretary, Dept. of Education and Science, P.O. Box 826, Canberra City, A.C.T. 2601, Australia; The Dept. of Health, Weilington, New The Canadian Medical Association, Alta Vista Drive, Ottawa 8, Ontario, Canada

### UNIVERSITY OF ST. ANDREWS DEPARTMENT OF GEOLOGY **BURMAH RESEARCH FELLOWSHIP**

FELLOWSHIP

Applications are invited for a fellowship supported by the Burmah Oil Company. The Fellow would be expected to initiate and sustain a project on the Solubility of hydrocarbons in water in a sedimentary environment in the Department of Geology. He should have had post-graduate experience in organic geochemistry and hold at least the degree of M.Sc. The appointment will be for free years in the first instance with a possible extension for a further two years. Salary scale £1,791 to £2,412 plus F.S.S.U and threshold arrangements.

Further information may be obtained from Professor E. K. Walton, Department of Geology, University of St. Andrews, Applications with the names of two referees should be sent to the Establishments Officer of the University, College Gate, St. Andrews, Fife, by December 31, 1974. (2063)

### CSIRO

### **AUSTRALIA**

# DIVISION OF PROTEIN CHEMISTRY

PARKVILLE, VIC.

### POSTDOCTORAL FELLOW

The Commonwealth Scientific and Industrial Research Organisation has a broad charter for research into primary and secondary industry areas. The Organisation has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

### FIELD

### PROTEIN CHEMISTRY

GENERAL: The Division of Protein Chemistry is located in Parkville, close to Melbourne University, and has a research staff of around sixty scientists. A wide range of physical techniques is available in the Division including an analytical ultracentrifuge; u.v., i.r. and fluorescence spectroscopy; e.s.r., n.m.r. and mass spectrometry; flash photolysis; spectropolarimetry. Comprehensive on-site computer facilities are also available. The research programme is concerned with the structure and chemistry of both animal and plant proteins but particular emphasis is given to the proteins of the wool fibre. Three distinct classes of proteins have been isolated from wool and representatives from each class have been purified and sequenced. During biosynthesis the wool proteins aggregate and interact in specific ways to form ordered fibrous assemblies which ultimately constitute the wool fibre.

**DUTIES:** To carry out a study of the properties of wool protein derivatives in aqueous solution and to search for evidence of specific interactions between proteins of the same class and of different classes. Purified proteins are available for this study and preliminary studies have shown that strong interactions occur.

**QUALIFICATIONS:** A Ph.D. degree, or equivalent qualification, in an appropriate discipline together with a demonstrated ability for original research. Experience in the fields of physical chemistry or physical biochemistry would be advantageous.

SALARY: The appointment will be made within the ranges of Research Scientist or Senior Research Scientist: \$A10,778 to \$A16,187 p.a.

TENURE: The position is available for a fixed term of three years.

Applications stating full personal and professional details, the names of at least two professional referees, and quoting Reference Number 462/393, should reach:—

The Personnel Officer,
Australian Scientific Liaison Office,
64–78 Kingsway,
LONDON WC2B 6BD

\*by December 27, 1974.

Applications in U.S.A. and Canada should be sent to The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W., WASHINGTON, D.C. 20036, U.S.A.

(2068)

# UNIVERSITY OF DEPARTMENT ANIMAL PHYSIC AND NUTRIT

Applications are invited fest of part-time RESEARCH FELL(tk on an A.R.C. funded project cone control of secretion of the gastr hormones and their significance iology of bovine milk fever.

Salary pro-rate on the 18 to £2,757.

Further particulars and at forms from the Registrar, The Unit Ledds, LS2 9JT, quoting reference 1/1/D. Closing date for applicatio-cember 2062)

### DRUMMOND FELLO® FOR RESEARCH IN NUON

RESEARCH IN NOTE

The Managing Trustees willer in May
1975 applications for a Drummlowship for
Research in Nutrition, normalite experience;
superannuation and expenses Preference
given to candidates with bioclinterests in
the field of inherited metabolis. Full particulars from: Honorary Sec Drummond
Trust, University College Londer St. London WCIE 6BT. Completed applications must
be received by March 3, 1975. (2065)

### ROYAL HOLLOWAYLEGE (UNIVERSITY OF LN) EGHAM HILL, EGHARREY RESEARCH STUDSHIP

Applications are invited graduates chemistry or physics for a restudentship study interfacial properties oded joints Marine craft. The successfulicant will expected to register for a higgree. Applications with curriculum vitae names a addresses of two referees she sent to P. J. Gardner. Department of points (N). (2069)

### UNIVERSITY OF IDESIA INSTITUTE OF MININGEARCH RESEARCH FELLCHIP IN APPLIED GEOCHSTRY

This fellowship is sponsoreothe Institute Mining Research and its objet is to estal the application element drainage reconnaissanarceys in the developed areas of Rhodesia is the sefellowship of a series, and threstigation consist of detailed orientarisurveys in part of Rhodesia, agst rocks ventain stratiform mineral dea. A trial, relement geochemical drainage will be and interpreted to demonstrate validity of technique. This study will comment the in gation of regional geochemical prints in own progress.

Applications are invited fi persons we

Applications are invited fi persons we good honours degree in geolo with a know of chemistry and an interest statistics. Fence in mineral exploration who has a day at it is expected that the results this investiful lead to the award of ther of Philic (minimum 2 years) and, depeng upon protection to be provided by the proposition of the provided by the provided by

Salary Scales: (Approx. Stequivs.): Juni search Fellow: £2.372 by £150 £2.828; Rt Fellow Grade II: £2.989 by £1 to £3.615. Salary according qualificatio experience.

Family passages and allowee for trans effects on appointment. Houg allowance, annuation and Medical Aid hemes.

Applications: Six copies, ving full paincluding name, place and ds of birth, to number, qualifications, experice, publication aming three referees, shod be submidecember 31, 1974 to the Assistant (Science), University of Rhæsia, P.O. E 167 Mount Pleasant, Salisbur, Rhodesia, P.O. Seption of the Association monwealth Universities (App), 36 Gordor London WCIH OPF, from whom condappointment may be obtaind.

### AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for appointment to the following:

RESEARCH SCHOOL OF BIOLOGICAL SCIENCES

### RESEARCH FELLOW-PARTMENT OF ENVIRONMENTAL BIOLOGY

A rellowship is available for research it one or both of the following areas:

(a) Plects of stomatal physiology; (b) Physiological ecology of stomatal behaviour. Applied have a Ph.D. in plant physiology or ecology and an interest in the appliciphysics and mathematics to biological problems. The appo. will have opport co-operate with staff working on related topics in physiology and tothe supervision of Ph.D. scholars.

Englid be addressed to Dr I R. Cowan, Department of Environmental Biological Sciences in the University.

Clos January 10, 1975.

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(2077)

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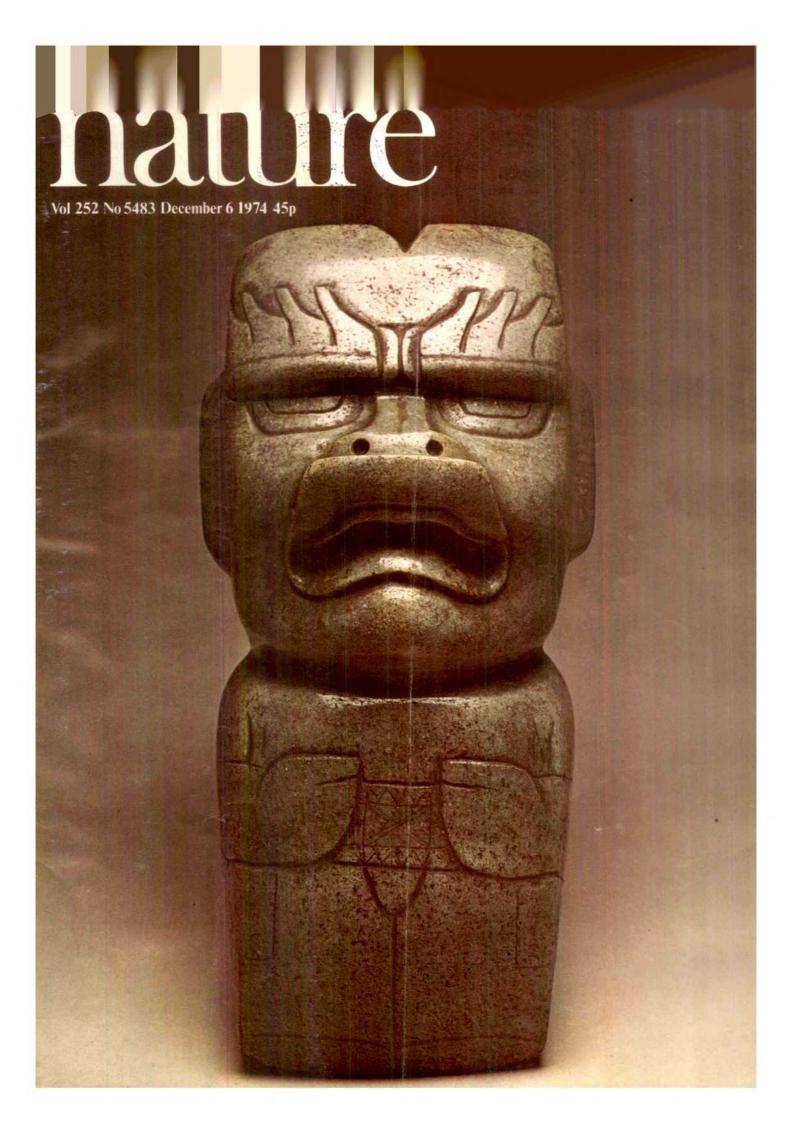
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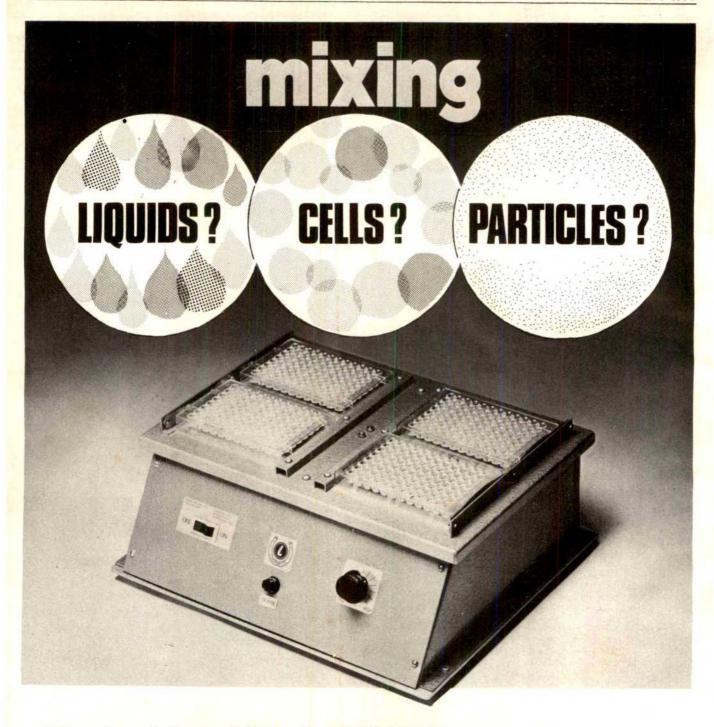
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Ceremonial axe of jade from the Olmec civilisation. An exhibit in the Museum of Mankind of the Department of Ethnography British Museum.

New radiocarbon dating of a Mayan site in Guatemala (p. 472) suggests that the earliest stages of the Mayan culture overlapped with the end of the Olmecs. (Photograph: Michael Freeman).



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December 6, 1974

### Limits to SALT...

EUPHORIA at the news that President Ford had achieved something in the way of a step forward in arms control during his recent meeting with Mr Brezhnev in Vladivostok has been short lived. Although it is certainly encouraging to know that Dr Kissinger has been able to persuade Mr Ford of the importance of keeping SALT high on the list of priorities, the expectations for the next round of talks are likely to be limited and, paradoxically, talk of a long control period (up to 1985) is bad rather than good news. The signs now are that SALT-2 will restrain both superpowers to do only what they intended to do in any case and will allow them ten years before they need to take up the running on limiting arms again.

SALT-2 froze for five years (1972-77) the total number of ballistic missile launchers at 1,710 (United States) and 2,350 (Soviet Union). Of these not more than 710 and 950 respectively could be submarine-based, and the conversion of land-based launchers from 'light' to 'heavy' character was prohibited. The figures were arrived at by little more than an examination of the *status quo* in 1972 and a belief that, if the numbers were disparate, quality made up for quantity. The ultimate in quality was, of course, to put many warheads on each missile, and the impossibility of satellite inspection of MIRVs ruled out any control of their extent.

Two years later the process of MIRVing is proceeding rapidly, with much of the United States fleet both at sea and on land already converted, and Soviet intentions (if lagging) perfectly obvious. Proposals for SALT-2 now include multiple warheads in the list of constraints, not, as far as one can gather, because of dramatic improvements in satellite inspection but presumably because it is considered economically desirable not to go the whole way on MIRVing.

Perhaps the most interesting development, however, is the agreement to trade off concessions. In return for acknowledgement that in SALT-2 strategic bombers will be included in the count of delivery vehicles (an American concession since their bombers out-number Soviet ones 3:1) the Soviet Union will not urge inclusion in the treaty of American forward-based systems. It seems that the total number of vehicles each side will then agree to keep to is about 2,500—a figure that the Soviet Union is already at and that the United States is close to. And 1,300 of these vehicles on each side would be fitted with MIRV, legitimising a total of nearly 2,000 more MIRVed missiles than exist at present!

The quantitative plateau on which both sides will happily take a rest is hardly likely to pose many problems of unemployment for the military-industrial complex. Accuracy and manoeuvrability have long since replaced size as the growth points of the missile field. Undoubtedly the next step is the MARV, or manoeuvrable re-entry vehicle, which can pursue a complex course

to its target, and no doubt sometime in the late 1980s when these have an accuracy of 10 metres or so, the superpowers will start talking about constraints on them.

### ... and UNESCO

Any discussion on the status of Jerusalem is bound to be charged with emotion, and anyone who chooses this particular instant to raise questions of the maintenance of the cultural heritage of the Old City must be assumed to be intent on making the maximum of mischief out of a complex situation. The sponsors, then, of the UNESCO resolution inviting the Director General to cut off assistance to Israel for its 'persistence in altering historical features' and undertaking excavations which endanger monuments 'subsequent to its illegal occupation of the city' were not acting out of purely archaeological concern. Whatever the rights or wrongs of the 'cultural' case against Israel there could hardly have been a more divisive time to raise this issue, and the sponsors. Arab and communist countries, are guilty of hypocrisy if they believe that the rest of the world will see their initiative as other than vulgarly political.

If the sort of moralistic criteria that they are applying to Israel were applied uniformly then Britain should be condemned for retaining the Elgin marbles, the Soviet Union and Czechoslovakia for severe restrictions on academic freedom, the United States for its past oppression of the Indian community and so on. Smart diplomats could rip apart UNESCO within months. But perhaps they need the contributions from the big boys more than they do from Israel.



THE TRANSIT OF VENUS

THE long-anticipated Transit of Venus took place yesterday morning; and already has the first instalment of news from distant observers arrived. The Astronomer Royal has been good enough to inform us that Col. Tennant's observations at Roorkee, India, have been quite successful; too photographs have been taken. He also telegraphs, at the moment of going to press, the gratifying intelligence that the micrometric observations near Cairo and Suez, and the photographic observations at Thebes have entirely succeeded.

At the last meeting of the Astronomical Society the Astronomer Royal gave an account of the final arrangements of the English parties, which do not vary much from those we stated some time ago. i Messrs. Green have arranged for one of their outgoing ships to pass near Kerguelen's Land, with a view of picking up intelligence and telegraphing it from Melbourne.

The southern stations occupied by the American, French, and German parties leave no doubt that the Halleyan method will be extensively employed.

From Nature, 11, 112, December 10, 1874.

### **CETI:** put not your trust in beacons

D. R. Bates, of the Department of Applied Mathematics and Theoretical Physics, Queen's University, Belfast, argues that it would be unjustified to devote resources to the interstellar communication problem on the assumption that there is a neighbouring technological civilisation which continuously operates a beacon signal suited to us.

THOSE active in promoting interstellar communication, for example through the \$10,000 million Project Cyclops, have faith that a neighbouring technologically advanced civilisation, X, would continuously operate a suitable beacon signal that, in addition to showing our civilisation (a technological novice) the star to which to send the acknowledgement signal, would carry other information. Most favour the beacon being omnidirectional because such a beacon by embracing all possible respondents within range, would avoid the contact-time difficulty.

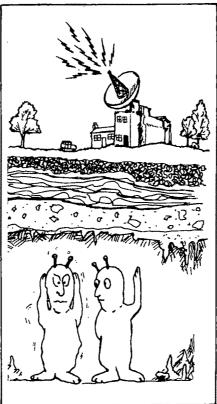
People are heedless to the needs of their descendants as their exploitation of the Earth's resources demonstrates. No terrestrial government would support a costly beacon if it were advised that a response (of debatable benefit) would be unlikely to be received for 200 years. In the case of a government on X, I shall quixotically suppose that the corresponding period is normally 1,000 years. This millennium criterion is a key factor. It would be imprudent for us to incur expenditure on the assumption that X, if indeed it exists, has a succession of governments with a multi-millennium criterion or with a sustained resolve to pass knowledge to alien species on distant planets.

On the millennium criterion the government of X would only finance a beacon if the probable distance to the closest potential respondent were expected to be less than 500 light years. From the spatial distribution of stars it follows that the number of potential respondents in the Galaxy would have to exceed the critical number

$$N_0 \simeq 5 \times 10^4$$

Drake's equation gives 
$$N=RL$$
 (2)

N being the equilibrium number of technological civilisations in the galaxy, R their annual rate of emergence and L their mean lifetime in years. The widely quoted estimate for R, 0.1 per



year, cannot be much too low. It may well be much too high: thus it rests on the dubious assumption that the abundant M and K-type stars are amongst the hosts and on the dogmatic assumption that the unknown probability of life developing on an apparently suitable planet is unity. Young technological civilisations (up to several times as old as us with age measured from the release of nuclear energy) are clearly so few compared with  $N_0$  that they would be dismissed as potential respondents on the millennium criterion.

Some may survive adolescent dangers and conceivably have lifetimes on the stellar evolutionary scale. Perhaps a sufficient fraction do so to make L long enough (at least millions of years) for  $N_c$  to be exceeded. Even granted this, a doubt remains. Ancient civilisations would have explored virtually all of science; they would have sophisticated historical records and rich cultural heritages to study. Many may lose interest in possible signals and even in astronomy, leaving the number in the communicative phase less than required.

Suppose that X has hitherto been isolated. Doubtless signal detection has been thoroughly tried: it has the attraction that its initiators might themselves have the satisfaction of witnessing success. Scientists of X could only

guess the probable distance to the closest potential respondent. Some might ask a government to finance a beacon project. For a government to spend unnecessarily on the basis of a guess would be strange. My quixotic supposition is that it does so provided the project satisfies the millennium criterion. In addition to the costs of constructing the beacon and associated power station the costs of maintaining and replacing them arise. Thought would therefore be given to the length of time for which transmission would be justified.

The number of advanced civilisations within the range of the beacon fluctuates around its equilibrium value. Such civilisations emerge at the annual rate

$$R_{\rm B} = R/N_{\rm C} \simeq 2 \times 10^{-8} R$$
 (3)

which is very slow. Hence the chance of getting a response would not be significantly increased by signalling for the full millennium rather than just for several decades. Several decades would suffice for the beacon to be noticed by any interested advanced civilisation within range and for much information to be carried by its signal (though whether or not this would reach another civilisation would be a dispiritingly open question).

If no replies came by the end of the millennium scientists on X would conclude that no potential respondents were within range. To increase the beacon's range ten-fold would necessitate increasing its power a hundred-fold. Scientists wishing this might attempt to find support for signalling efforts which at best might be acknowledged between 1,000 and 10,000 years later. We would be prodigal to divert resources from promising research on the assumption that they would succeed.

If replies to the first beacon came, the triumph would not make it much easier to persuade a government of X to finance a second. A better case could be made for financing a brief beacon project (covering hitherto unexplored stars) by a government of one of the respondents not already in contact with other civilisations.

Should a communication cell once be established, its radius might expand at a speed comparable with that of light until the entire Galaxy were occupied. For this to be possible the total number of civilisations in the communicative phase would have to exceed  $N_c$ , which is large. Because of the slowness of the emergence rate given

by equation (3), it would be improvident for us to proceed on the assumption that a government of a civilisation belonging to the hypothetical communication network would finance a prolonged beacon project aimed merely at hastening contact with any relatively recently developed civilisation rather than leaving the initiative to the newcomer as the one having the greater incentive and the easier problem.

The inference that it would be irresponsibly optimistic to assume that civilisations operate beacons for more than minute fractions of their lifetimes in the communicative phase has an important consequence. It follows im-

mediately from Drake's equation that relatively few beacons can realistically be expected to co-exist. A proper allocation of research effort cannot ignore that we are hence probably much farther from the nearest beacon than the beacon is from its intended respondents. The acute difficulty this causes is greatly aggravated by another factor.

The communication techniques of a civilisation initially improve rapidly but improvement cannot continue indefinitely. Instead the rate of improvement must decrease and eventually become vanishingly small. Presumably all civilisations which survive long enough

reach a common level of technical attainment, and presumably most beacons are designed by and suited to those at or near this common level. A comparison between Projects Ozma and Cyclops shows strikingly how rapidly our techniques are still improving. The pattern of our development can scarcely be such that Project Cyclops, though far ahead of Project Ozma, is close behind what may ultimately be achieved. Yet, unless the pattern is indeed such, we must be technically much too backward for it to be worth our while engaging in the basically extremely formidable task of searching for signals. 

# international news

PERSISTENT reports of the arrest. torture and execution of doctors and other health workers have been filtering out of Chile ever since Salvador Allende's government was overthrown by the armed forces last year. Although the military junta has consistently denied such charges, most conspicuously in a full-page advertisement in the New York Times, an investigation by three biomedical scientists with support from the Federation of American Scientists (FAS) has now accumulated considerable evidence that left wing members of the medical profession have indeed been a prime focus for particularly brutal reprisals since the junta seized power.

Most of their findings simply back up many previous allegations of brutality, but the report breaks new ground with its analysis of why doctors in particular were singled out for harsh treatment and it also has some disquieting things to say about the Colegio Medico—the Chilean equivalent of the British Medical Association.

First, the Colegio played an active part in Allende's downfall by organising two damaging strikes, one in 1972 and the second immediately before the coup, and it issued two demands for Allende's resignation. After the armed forces seized power and began rounding up Allende's supporters, however, the Colegio did nothing to defend the rights of any of its members, even though its own code of ethics states that it is obliged to defend accused physicians. As Dr Leonard Sagan of the Palo Alto Medical Clinic, who led the investigation, puts it: "the Colegio took almost a brutal delight in the overthrow (of Allende) but it had no compassion whatsoever for its members who were arrested".

### Reprisals against Chilean doctors

by Colin Norman, Washington

In particular, the report notes that one doctor, Carlos Shuster, wrote a letter to the Rector of the University of Chile suggesting that all physicians be classified into three groups "those above suspicion, those with leftish sympathies and those beyond rehabilitation". That letter "seems to have suggested to authorities a general system for classification of health workers into A, B, and C lists", the report suggests, and those lists later served as a basis for arrests and other reprisals.

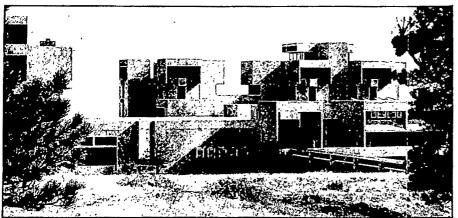
Local responsibility for compiling the lists fell to directors of hospitals and chiefs of service, the report states, and "while we were unable to obtain direct evidence, it seems probable that officials of the Colegio Medico, on the national and local levels, were involved in preparing lists of physicians considered not only dangerous but also politically unacceptable". It adds, however, that only in the Medical School of the University of Chile "did we hear, from the dean, a clear distinction between ideological allegiances and participation in violence. The dean asserted that, at least within his jurisdiction, this distinction has been respected."

Appearance on a list had a variety of consequences. "In some cases it appears that certain physicians were marked for death", the report states; others were arrested and several have been barred from working for the health service—which is tantamount to denying them a job at all.

The investigating team says that it has "good evidence" of 176 physicians who have been suspended from the health service, and it has a list of 109 physicians who had been imprisoned up to June this year. Dr Sagan said last week, however, that the figure for total arrests of doctors is probably nearer 300. In addition, some 250 doctors have fled the country since the country.

As for reports of executions, the team arrived in Chile with a list of 28 doctors believed to have been shot, but several of the reports turned out to have been unfounded. They found good evidence, however, that at least fifteen doctors have died since the coup, and "of those deaths for which we have relatively reliable information, death occurred shortly after arrest in the month of September". The most common excuse given by the authorities was that prisoners were shot while trying to escape, but the report states that such a story is implausible at best.

Finally, the report notes that allegations of torture are widespread, but "are difficult or impossible for foreigners to substantiate in a charged political environment". Nevertheless, the investigating team repeatedly heard two disturbing allegations about the source of the methods of torture used. First, a report that a group of Brazilians were brought to Chile to train the Chilean military was "widely heard" and, second, "there are frequent references to an American training camp in Panama where South American military officers are indoctrinated in techniques of resisting torture, thereby providing technology on the information of torture".



# Trends in meteorology

by John Gribbin

THE National Center for Atmospheric Research (NCAR) in Boulder, Colorado, has just issued its annual report for 1974. The NCAR was set up in 1960, and is run by the University Corporation for Atmospheric Research (UCAR), a private, non profit corporation whose 44 members are universities and other institutions with doctoral programmes in the atmospheric sciences. The operation of the NCAR is supported by the United States National Science Foundation; in practice, this means that the NSF runs the NCAR for the UCAR on a contractual basis. The Center is unique—there are only four other national centres run by the NSF and all of those are centres of astronomy. Indeed the NCAR is itself responsible for the High Altitude Observatory (HAO) but this is not as odd as it might seem, since that observatory carries out research on the physics of the Sun and the solar terrestrial environment, areas of astronomy which seem to have great relevance to problems of the behaviour of the Earth's atmosphere.

Public, and indeed journalistic, attention has focussed on centres like the NCAR lately because of the increased awareness of the problems of climatic change. But as the new annual report shows, this kind of work has been somewhat out of the mainstream of research at the NCAR. That situation may now be changing but more traditional aspects of atmospheric research are clearly still at the forefront as yet.

Topics covered in the past year include research into storm forecasting, the investigation of the interaction of aerosol particles with liquid and ice constituents of clouds, and the airflow and moisture budget of a hailstorm. Some of the work on mechanisms of hail growth highlights just how great the problems of understanding all the workings of our atmosphere are—some theories of hail formation postulate that hail growth begins with the freez-

ing of large supercooled water droplets; others argue that it begins with ice riming around individual ice cyrstals. Studies at the NCAR show that, for Colorado storms, growth from the ice phase is indeed dominant; but Soviet workers report great success with the alternative model. As the authors of the report put it "geographic and en-

### Cloud research

PROFESSOR Jim Megaw, of York University, Toronto, says that as far as man's influence on climate is concerned "the time for descriptive work is past". Like many other people he is greatly concerned about the effect of freon on the ozone layer and believes that its use as a propellant in spray cans should be stopped. Another problem which needs urgent attention is the growth of industrial clouds from the smoke and gas emitted by factories; here, Megaw is at least on the trail of a technique which would make possible the destruction of man's influence on the environment at source.

It seems that laboratory experiments on cloud formation have from time to time been hampered because the 'clouds' refused to form in the cloud chambers. The blame for this has now been laid at the door of the pure ion exchange water used by the experimenters. There is something in ion exchange water which can inhibit the formation of mist, clouds or fog, and it seems very likely that the elusive 'something' is an amine (one of the same family of compounds as those which give fish their characteristic odour). Megaw is now trying to identify the exact compound involved, which is effective in tiny quantities. If it can be pinned down, it could be added to industrial products to inhibit formation of industrial mist and fog. Although Megaw did not mention the possibility of use in aircraft, this would also seem an ideal way to reduce or remove the hazard of increased cloud cover produced by high flying jets.

vironmental differences may provide some explanation of the discrepancy", but if both theories can be correct, depending on circumstances, then a real understanding of hailstorms is far from being at hand.

If that is the case with something as seemingly simple as a hailstorm, the job of those working on climatic modelling might seem impossible. But progress is being made, at the NCAR and other centres. One study of the effect on surface temperatures which might result from changes in stratospheric aerosol or ozone concentrations brought about by operating a fleet of supersonic transport (SST) aircraft seems likely to fan the flames of the SST controversy once more. The effect of the increased stratospheric aerosols on the NCAR model is a reduction in surface temperature by 0.25 K, whereas the effect of the nitrogen oxides emitted by the hypothetical SST fleet is a rise in surface temperature by as much as 1 K. In the first case, increased reflection of solar heat dominates; in the second there is a net increase in absorption. These studies offer no easy solution to the question "is an SST fleet environmentally safe?" but since a change in surface temperature of 0.1 K is considered by the experts to promise significant climatic effects it does seem that the question should at least be asked once again before SST operations begin on a large scale.

Studies of the feedback between temperatures and cloud cover also show how climatologists must solve the chicken-and-egg problem. We hear a lot about how increased cloud cover can cause more reflection of solar heat and a fall in surface temperatures. On the other hand, preliminary results of studies now continuing at the NCAR indicate that there is a positive feedback between changes in surface temperature of the sea and the cloud cover.

The solar studies of the HAO also extend to include work on Skylab, through the Apollo Telescope Mount, and the Executive Director of the NCAR, John Firor, sees studies of the Sun as of increasing importance to problems of climate and atmospheric modelling. Such statistical correlations as there are between solar phenomena (notably the sunspot cycle) and terrestrial weather have not been explained by any satisfactory physical model, but they have encouraged a new look at the solar influence. Indeed, now that the astrophysicists have been forced, by the absence of detectable solar neutrinos, to accept that they do not know all about the workings of the Sun there is room for climatologists to question just how constant the 'solar constant' radiation reaching the Earth is.

research programmes ever undertaken Directors. -the joint French-American explora-Because of a chronic shortage of funds Alvin, which is reckoned to be the best his election statements. of the few research submersibles operated in the United States, would be mothballed.

But last week three federal agencies announced that they have joined forces to keep Alvin operating for at least three years. The National Oceanic and Atmospheric Administration (NOAA), the National Science Foundation (NSF) and the Navy will each put up \$300,000 in 1975. They will renegotiate the funding levels for 1976 and 1977 on an annual basis, but it is estimated that about \$1 million will be found for each of those two years.

The new funding arrangement, apart from being welcome news to Alvin's research crew, is something of an experiment. The NSF has supported Alvin's work in the past on a projectby-project basis, but according to one NSF official, "we would now like to see what the scientific demand will be with a stable funding level". Alvin's scientific programme for next year is now being worked out.

American Chemical Society (ACS) opted for a decisive shift in the ACS's activities when they elected Alan Nixon as the society's president. Nixon conducted a vigorous election campaign (which itself was without precedent in the society's affairs) based on the promise that he would make the ACS more responsive to the professional needs of its members. And he has been followed by two like-minded presidents — Bernard Freidman this panded such activities as manpower cessful sabotage". surveys, public information and Conthe legal expenses of its members who are in dispute with their employers.

Glenn T. Seaborg, a former Chairman terrorist. of the Atomic Energy Commission and

the Woods Hole Oceanographic Insti- cies for the ACS came in third. More- Atomic Energy Commission itself is tution) returned this summer from over, Nixon himself failed in a bid to sponsoring a number of studies aimed one of the most successful undersea be elected to the ACS Board of at defining the level of risk and the

tion of a section of the Mid-Atlantic generally viewed as a break with the pleted in July next year. Ridge—it faced an uncertain future. tradition established in the past few



Washington seen

by Colin Norman

• The General Accounting Office (GAO), an investigative arm of the United States Congress, has carried out a survey of security systems at nine • Having recently banned the manunuclear power stations in the United facture of the pesticides aldrin and States, from which it has concluded that they are probably ineffective in Agency (EPA) is now moving against preventing reactor sabotage aimed at two more widely used insecticides, • Three years ago, members of the releasing large quantities of radioactive chlordane and heptachlor. Last month material into the environment.

leased last week by Senator Abraham registration of those two pesticides-Ribocoff, contends that "a security sys- an action tantamount to removing tem at a licensed nuclear power plant them from the market-because they could not prevent a takeover for may be carcinogenic. sabotage by a small number—as few, perhaps, as two or three—of armed tachlor were used in 1972 on corn, individuals". It adds that "such a take- vegetables, cereals, forage crops, seed over, particularly of a nuclear power crops and seed treatment, and about plant near a large metropolitan area, 16 million pounds of chlordane were could threaten public health and safety used to control fruit and vegetable year and William Bailey next. The up- if radioactive materials were released pests and for various household and shot is that the ACS has greatly ex- to the environment as a result of suc- commercial uses.

didate added to the ballot by chemists saboteur did manage to gain access to stillborn infants.

WHEN the submersible Alvin (based at who generally supported Nixon's poli- the reactor site. Consequently, the possible consequence of successful Even though Seaborg's election is sabotage. The studies should be com-

The GAO points out, however, that years, it should be noted that he at at least one part of a power plant for submarine research, Woods Hole least paid lip service to the need for seems to be both accessible and vulofficials were then saying privately that the ACS's professional programmes in nerable to sabotage—the facilities used for temporary storage of used fuel

> In the United States, when fuel rods are removed from the reactor core they are placed in an uncovered pool of water near the reactor for cooling with the result that "they do not have the same degree of physical protection as that provided to the reactor core by the reactor containment vessel".

> The spent fuel rods are only supposed to be stored at the reactor site for a short time before being packaged and shipped to reprocessing facilities. But the problem is that no commercial reprocessing facilities are yet operating in the United States, so that more used fuel is now on hand at power plants than normal. The GAO therefore recommends that interim security arrangements should be established immediately to protect this material.

dieldrin, the Environmental Protection EPA Administrator Russell Train an-The GAO's report, which was re-nounced that he intends to cancel the

About 3 million pounds of hep-

The EPA has decided to move In particular, the GAO found that against the pesticides because of a feedgressional liaison, and it has even in several of the plants it visited there ing study which has shown that heprecently set up a loan fund to help with were breaches of security regulations tachlor and its metabolite significantly such as unlocked outside doors, lack of increase the incidence of liver tumours intrusion alarms, unarmed watchmen in one strain of mice at the level of 10 But there are signs that the society and unlit proteoted-area perimeters— parts per million in the diet, a feeding may now be opting for a change back security measures, in other words, study with rats which showed up a to its more traditional style of leader. which would pose little problem for an tumorigenic effect at intake levels as Last month the members elected amateur burglar let alone an armed low as 0.5 parts per million, evidence of embryotoxicity from both hep-Be that as it may, there is consider- tachlor and chlordane in some strains a nuclear chemist with impeccable able disagreement within the nuclear of rats and mice, and the discovery in academic credentials, to be president industry about the vulnerability of hospitals in Atlanta of heptachlor of the ACS in 1976, whereas a can-nuclear reactors to sabotage even if a metabolites in tissue taken from 10

# Heavy water shortage faces India

from Narender K. Sehgal

In less than two years' time, India is likely to find her heavy water requirements exceeding availability. So, what's new? Nothing except that, unlike other items, heavy water is a 'nuclear' material and its import from abroad may involve more than mere commercial considerations. (Heavy water is used as moderator and coolant in the Indian nuclear reactors.) In the wake of recent developments following India's explosion of a nuclear device, nuclear powers such as the United States, the Soviet Union and the United Kingdom along with Australia, Canada and West Germany are reported to have decided that no vital 'nuclear' materials shall henceforth be supplied to non-nuclear weapon countries unless they are subject to International Atomic Energy Agency (IAEA) safeguards under an agreement. It is not yet clear whether or not this implies that these countries will refuse to sell heavy water to India. The IAEA meeting at Vienna recently did not provide a clear cut answer.

Work is now in progress in India on three nuclear power projects, one each in Rajasthan, Tamilnadu and Uttar Pradesh. (One of the units of the Rajasthan project is already delivering power. At present, India has a small heavy water plant (14 tonnes annual capacity) at Nangal in Punjab. But four more plants with a total annual capacity of some 300 t are under construction at Kota (Rajasthan), Baroda (Gujarat), Talcher (Orissa) and Tuticorn (Tamilnadu). The production will reach maximum capacity in stages and only by 1978 will it near the 300 t mark-that is, if work goes on as planned.

According to Atomic Energy Commission calculations, India will have to find something like 200 t of heavy water from outside sources during 1976-77 and then again during 1981-83 when shortfalls will occur if no additional production capacity is installed.

In theory, design specifications provide 100% leak-proof heavy-water systems in reactors and after the initial loading (which means more than 200 t of heavy water for each of the reactors under construction in India) no more should be required. But in practice leaks invariably do occur during operation and arrangements are always made to recover the leaked fluid and upgrade it for reuse. However, some replenishing is always needed to make up for losses during the recovery process.

India is reportedly already negotiating with several countries in this con-

nection. If the countries mentioned above choose to enforce their decision, France may be the only alternative left. Then again, the question will hinge on whether or not France will be able to spare the amounts of heavy water required by India, after her own needs are taken care of. If it does not become possible to arrange the needed quantities of heavy water, the already bad power situation will worsen further as the nuclear power projects will either face delays or curtailments in power generation, or both—a price India may have to pay for her two 'sins': conducting a nuclear explosion and refusal to sign the nuclear non-proliferation treaty!

# Conservation golden jubilee

from Vera Rich, London

November 29, 1974 marked the golden jubilee of the All-Russian Society for Nature Conservation, an organisation which has grown over the years to a membership of some 26 million, and which has as its chairman no less a personage than the Deputy Minister of Land Reclamation and Water Economy of the Russian SFSR, N. G. Ovsyannikov. Accordingly, in addition to the official notice of the anniversary, which reported such diverse activities as reafforestation, the protection of beavers, the study of the ecological effects of damming rivers, and the propagation of the correct Leninist attitudes to nature, Pravda has also carried a number of other news items slanted towards conservation interests.

Thus, on November 20, the "temporary rules" for the conservation of Lake Baikal were announced. These rules, confirmed by the Ministry of Land Reclamation and Water Economy after a long and careful study are aimed primarily at preserving the water resources of the lake. In addition to the purification measures already introduced for industrial plant (especially paper and timber mills) on the tributary rivers, the new rules envisage the maintenance of the rivers themselves. All submerged timber is to be removed from the river beds, and the rafting of timber down these rivers is to be stopped entirely. The transportation of timber by means of "water-stable floats" is to be extended. All waters of the Baikal basin and of the lake itself are to be protected from physicochemical and biological pollution of all kinds—and, by means of careful organisation and control of holiday centres, sanatoria and rest-homes, from tourist pollution as well. And, in accordance with the Siberian proverb "While there's forest, there's Baikal", all forestry operations in the area will be carefully monitored to obviate erosion and drying out of the soil, with, in particular, a total prohibition on all tree-felling on slopes with a gradient exceeding 15°.

Again on the subject of forestry resources, a report from Kiev (Pravda, November 21, 1974) notes that in the rice-growing areas in the south of the Ukrainian SSR more than 250,000 tonnes of rice straw go to waste each year. In the very same region, there are two paper and cellulose combines which every year consume up to 300,000 cubic metres of timber, brought from the Arkhangel'sk region on the White Sea. If the rice straw, already on the spot, could be used instead of timber, not only would it conserve several thousand hectares of forestland per year, but would also save the cost and logistics of shipping the timber from the extreme north to the extreme south of the USSR.

"conservation" of human The ecology from natural disasters is also well represented: on November 24, a report on measures against erosion by wind and water in the Caucausus, and Transcaucasia, Kazakhstan, the Urals and Eastern Siberia; on November 27, a report from a hydrotechnical research expedition to the Amu-Dar'ya, the "most capricious" of Soviet rivers, where there is a unique problem of bank erosion, locally known as the Deigish, which can lead to extensive flooding, with destruction of hydrotechnical installations, fields and plantations.

And from Vladivostok, in the interests both of conservation and ecology, an interview with Vladimir G. Osipov, Head of the Sector of Oceanic Pelagic Fish of the Pacific Scientific Research Institute of Fisheries and Oceanography, explains the current interest of his Institute in sharks. Not only are they of interest to the ocean ecologist, standing as they do at the apex of the "food pyramid", and to the palaeoichthyologist-they also represent for mankind "a considerable reserve of protein". Shark's liver is rich in vitamins, and the medical industry has been interested for a long time.

Unfortunately, there remains the hazard to life and limb of those deputed to study and catch sharks. Although shark-repellent substances, electrified barriers, or, "most promising", special nets, can be used to protect bathing beaches, it is clearly impossible to study sharks at long range, and "scientists have recorded a large number of tragedies" while working in this field. Like many ecological and conservation problems, shark harvesting seems a matter of theoretical promise but great practical difficulty.

# correspondence

### Irrationalism and science

SIR,—It is no longer surprising to find scientists consulting the stars or the I Ching to help make decisions, but it is nonetheless depressing. It is a sign of the times—a pandemic disillusionment with political and social institutions. with the past, present and even the future. In its wake, rationalism hides defensively in a few remaining outposts, while mysticism strides about in its various forms, pointing accusing fingers at the anti-human, anti-God, anti-fun, anti-truth, anti-soul, anti-life of science and rationality. Like all trends, irrationalism is not new, but the speed at which it has come upon us this time around and its proportions are a little frightening.

Uri Geller is a case in point. Ten years ago he would have been a charismatic illusionist; today he is a cult figure, believed by many to have various super-human powers normally reserved for Gods and comic-strip heroes. Scientists join him on television programmes and assure us that his feats cannot be explained by present-day science, neglecting the possibility that they may be best understood in the context of present-day magic. That television, radio and newspaper science journalists should join, or even lead the parade of the occult is perhaps not surprising, but that a prestigious scientific journal like Nature should be a credulous participant is a disquieting indication of how far irrationalism has invaded our profession. By acclaiming Geller an important "challenge to scientists" (Nature, 246, 321; 1973) and by publishing an inadequately controlled study on Geller's performance at the Stanford Research Institute (Nature, 252, 559; 1974), Nature has added its prestige to irrationalism and given it a coveted stamp of scientific approval. Rather than making exceptions and lowering standards in order to publish papers of this kind, surely scientists, and the journals that represent them. have a responsibility to themselves, to science and to society to defend the rational approach against the present wave of obscurantism and anti-reason. Although this will raise cries of "scientific elitism", it is simply recognising the definition of science, and approach does not demand the disclaiming of unusual phenomena as impossible, but rather an objective assessment of the probabilities in explaining them, and excluding all natural explanations before turning to supernatural ones. To do otherwise is irrational. A rational perspective on Uri Geller was provided by the *New Scientist* (October 17, 1974).

No matter what the explanations for Geller's various feats turn out to be, he has served to point up two wellknown, but often forgotten facts that deserve publicity and further study. People are remarkably inaccurate observers and reporters of events, even when their professions, such as science or journalism, rely heavily on objective reporting. Suggestion can have powerful effects on the thoughts, sensations and behaviour of most people, which can be useful (as in acupuncture anaesthesia), but which also can be dangerous. It is sobering to think what might happen if anyone believed to have Geller-like powers decided to prognosticate on political or economic issues.

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### Pacem in maribus

SIR,-Wendy Barnaby's otherwise fair account of the Pacem in Maribus Convocation (Nature, October 11) gives the impression that I said the "nonconventional" living resources of the ocean-such as squids, krill, lanternfishes—are found mainly in the area beyond the proposed Exclusive Economic Zone. That is not so. The large potential food resources are found both far offshore and rather near coasts. Their geographical distribution is not well known, but the occurrences of high densities are related to zones of high primary productivity. Uncertainty as to jurisdiction over these resources derives less from ignorance of the distributions, than from uncertainty as to the extent of the coastal regime yet to be negotiated. There are many areas of controversy regarding the zonal concept and estimates of the proportion of the total ocean surface which would be under some form of coastal state jurisdiction range from 30 to 90%.

The "unconventional" resource nearest to commercial exploitation is the krill, much of which occurs within 200 miles of the Antarctic continental coastline. With the present Antarctic Treaty in force there would presumably be no EEZ claimed there, but some Parties to the Treaty are considering possible revisions to it to facilitate resource exploitation (*Science*, 184:776–780; 1974). Of the 12 Parties only two are "developing" states. Seven of the remaining ten are affluent northern hemisphere nations, and three of them have active programmes of research and development on krill.

In future either much of the kill resources will be in the waters of the "international zone" or "high seas" or in the coastal zones of a few countries, including perhaps those which successfully lay claim to the Antarctic resources. Similar arguments can be made for other unconventional resources elsewhere. It may be unwise for the developing countries to assume that the potential living resources of the ocean will lie, as do those resources which are now exploited near to their biological limits, mainly within the jurisdictions of coastal states under an EEZ regime. Further, to which particular types of resources (apart from minerals) such iurisdictions will apply, and under what restraints, remains to be determined, with many difficult questions as yet unresolved.

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### Beware the ghost writer

Sir,—One may sympathise with Dr Cater (Nature, November 29) who has had his work attributed to Dr Carter but what of the attribution of work to authors who do not exist?

In the journal Cancer (29, 1398; 1972) appears a paper by T. Ghose and S. P. Nigam but unfortunately one of the qualifications following Professor Ghose's name is given as M.R.C.PATH and is printed in the same fount as the authors' names.

This has confused several writers quoting this paper who have referred to it as being by Ghose, Path, and Nigam.

The phantom Dr Path has unfortunately been given bibliographical clothes by the cancer abstracting journal Excerpta Medica (Cancer Section) (23; 1973) who give him an entry in their author index.

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# news and views

### A new stable particle: is charm appearing?

INTENSE interest has been aroused by the observation of a new particle with a mass of 3.1 GeV/ $c^2$ . Claims from Brookhaven, New York, where it is called the J meson, and from Stanford, California, where they call it the  $\psi$ , agree so far on its major physical properties. The Massachusetts Institute of Technology group led by Sam Ting has seen a very sharp peak in the mass spectrum of e<sup>+</sup>e<sup>-</sup> pairs, produced when a beryllium target is exposed to the 29 GeV/c proton beam from the Brookhaven Alternating Gradient Synchrotron. The peak is only about 5 MeV/ $c^2$ wide, which is consistent with the resolution of the apparatus, and its production probability is about 10<sup>-7</sup> times the probability for producing strongly interacting particles (hadrons). A Stanford-Berkeley group led by B. Richter used the Stanford electron-positron storage rings 'SPEAR' to produce the new particle directly by e<sup>+</sup>e<sup>-</sup> annihilation. They claim that its width is less than 1.3 MeV, and that it decays to hadrons, to e<sup>+</sup>e<sup>-</sup> and probably to pairs of muons. In contrast to the low relative probability for producing the particle with protons, the electron-positron interaction probability is enhanced by a large factor as a consequence of  $\psi$  (or J) production.

It is clear from the way that the particle is produced that it does not couple strongly to the normal hadrons—the proton and neutron, the hyperons, the pion and kaon, and so on. If it did, then its production rate at Brookhaven should have been much greater. The relatively large production rate at Stanford implies that the  $\psi$  (or J) couples to e<sup>+</sup>e<sup>-</sup> by a simple (lowest order, single photon) electromagnetic process. Weak interactions, as understood at the moment, could not account very easily for the Stanford results.

A particle without strong decays is usually regarded as 'stable', and a width of less than 1.3 MeV/ $c^2$  implies a lifetime of more than  $10^{-21}$  seconds—a long time on the strong interaction scale. If the  $\psi$  (or J) carries only known quantum numbers then it is very hard to see why it should be stable. With 3 GeV of decay energy available it is possible to imagine hadron final states with almost any combination of quantum numbers. As long as such final states can exist, then we would expect strong decays of the  $\psi$ . Electromagnetic decays do not conserve as many quantum numbers as strong decays do. That is why the well established eta meson can decay electromagnetically into three pions, for instance, even though the conservation laws forbid a strong transition from eta to three pions.

Three of the possible explanations for the  $\psi$  deserve mention. The obvious first hypothesis is that it carries a new quantum number which is conserved by strong interactions but is not conserved by electromagnetic processes. This cannot be ruled out, but it is an uneconomical theory since it requires a totally new quantum number to be invented specially to explain this one finding. A second hypothesis is that the  $\psi$  is the intermediate boson which carries the weak neutral current (the intermediate vector boson is a heavy particle, probably as heavy as 40 proton masses, which carries the weak interaction; see Nature, 250, 186; 1974, for the present status of experiments on weak neutral currents). But if the boson mass is only

3 GeV/ $c^2$  we would expect very large differences between the properties of the neutral currents as observed in the CERN Gargamelle experiments with 5 GeV neutrinos and in the Fermi Laboratory experiments with 100 GeV neutrinos. No significant differences have been seen. The third and most elegant hypothesis also incorporates a new quantum number, but one which has already been suggested to explain the absence of strange particle production in neutral-current processes. The  $\psi$  may be inhibited from strong decay because of 'charm'; not because the  $\psi$  itself is a charmed particle, but because it is made of a charmed quark and a charmed antiquark.

Another well established meson, called the phi, also has electromagnetic decays to e+e-. It is not quite a stable particle—it decays by the strong interaction to a kaon plus an antikaon-but it chooses not to decay by the far more accessible channel to three pions. The omega meson, which has all the same normal quantum numbers as the phi, and is lighter than the phi, decays very rapidly to three pions. The absence of a phi to three pion decay has been explained very convincingly by saying that the phi is made from a strange quark and a strange antiquark. In a decay process the strong interaction apparently preserves the quarks that were contained in the decaying particle. The lightest particles which contain strange quarks are the kaons, so the phi either decays to a kaon-antikaon pair, or it decays electromagnetically. If the kaons just happened to be about 10% heavier than they are, then the phi would not be able to decay into them—its mass is 1.018  $GeV/c^2$  and a kaon has a mass of about 0.49 GeV/ $c^2$ .

Perhaps the  $\psi$  is a similar object to the phi. Charmed mesons may exist, like the strange mesons we call kaons, but if the lightest of them is more than half the mass of the  $\psi$  then the  $\psi$  will not be able to decay into a pair of them through the strong interaction.

D. J. MILLER

# Misunderstandings over C<sub>4</sub> carbon fixation

Ecologists concerned with energy flow in ecosystems, and particularly those interested in primary energy fixation, await a clarification of the status, both in energetic and ecological terms, of the recently described C4 pathway of carbon fixation found in certain angiospermous plants. At present the literature presents a rather confused picture. Statements now proliferate which give the impression that there are "two major photosynthetic pathways of carbon assimilation in higher plants" (quoted from Osmond, Aust. J. Bot., 22, 39; 1974). Although such authors, and perhaps the initiates among their readership, may have a clear idea of what this means, to those unfamiliar with the biochemistry of these photosynthetic processes (which probably includes the majority of ecologists) the implication is that the so-called C3 and C4 plants have distinct and dissimilar methods by which carbon is assimilated into the plant tissue. In fact this is not quite true. Although C4 plants possess a novel system for carbon concentration in

which carbon dioxide initially reacts with phosphoenol pyruvate to form oxaloacetate (a four-carbon acid), a second fixation process is necessary prior to the assimilation of the carbon into plant tissues and is achieved by a release of CO<sub>2</sub> from four-carbon acids within the bundle sheath cells, where it is refixed in the chloroplasts by the conventional C<sub>3</sub> mechanism. This involves ribulose diphosphate (five-carbon) and the production of three-carbon molecules (Hatch, in *Photosynthesis and Photorespiration*, edit. by Hatch, Osmond and Slack, Wiley, New York; 1971; and Black, A. Rev. Plant Physiol., 24, 253; 1973). A major significance of the C<sub>4</sub> process is its capacity for operating at very low ambient CO<sub>2</sub> concentrations, when it serves to concentrate CO<sub>2</sub> in the mesophyll cells before passing it on to the bundle sheath cells.

The confinement of the Calvin cycle to the chloroplasts of the bundle sheath cells is by no means proven. Evidence for this is mainly negative in that ribulose diphosphate carboxylase activity has not yet been demonstrated in the chloroplasts of the mesophyll cells, whereas posphoenol pyruvate carboxylase has been shown to be active in the mesophyll cytoplasm. The precise location of the Calvin cycle is still in dispute and some people are inclined to believe that it may occur in the mesophyll (see Coombes, Commentaries in Plant Science, 1, 1; 1973, in Current Advances in Plant Science).

Evidently the initial stage of the  $C_4$  system of carbon concentration is not an alternative to the Calvin cycle  $(C_3)$  system, but is an additional mechanism which can be advantageous in certain environmental circumstances. In many respects it is comparable to the crassulacean acid metabolism of certain succulents. (Laetsch, Am. J. Bot., 55, 875; 1968; also see Nature, 251, 380; 1974).

Another area of misunderstanding is the higher photosynthetic rate and, in some cases, higher primary production rate associated with the C<sub>4</sub> mechanism (Zelitch, Photosynthesis, Photorespiration and Plant Productivity, Academic Press, London and New York; 1971). Many C4 species, such as maize and sugar cane, are among the most productive in the world, and this has led to the assumption that the adaptive significance of the C4 mechanism is one of enhanced productivity. Gifford (Aust. J. Plant Physiol., 1, 107; 1974) has recently examined this concept by considering the relative advantages of the C, system from the molecular to crop growth rate levels of organisation. At the level of initial CO2 reaction, the C4 mechanism may have a 60-70fold advantage (but this is questionable in the light of Bahr and Jensen's work: Plant Physiol., 53, 39; 1974) over the C<sub>3</sub> system, due mainly to the much lower CO<sub>2</sub> concentrations at which it is able to operate. Consideration of leaf mesophyll in vivo shows that this advantage may have fallen to about five-fold at the tissue level due in part, perhaps, to the necessity for refixation in C4 plants.

A comparison of daytime net photosynthesis of  $C_3$  (for example ryegrass) and  $C_4$  (for example maize) crops with similar leaf area indices and under the same growth conditions shows that the  $C_4$  species may have a 1.5-fold advantage at high light intensities, but this declines with lower light availability. If crop growth rates are measured rather than gas exchange under idealised conditions then one finds considerable variation among both  $C_3$  and  $C_4$  species but there is no evidence for consistently higher growth rates among  $C_4$  plants. Gifford concludes that some of the very high forage yields reported from some tropical  $C_4$  grasses, such as *Pennisetum*, may be related to the greater length of the tropical growing season rather than the higher short-term production efficiency of these species.

Evidently there has been some oversimplification regarding the potential advantages of the C<sub>4</sub> sytem; high efficiency at the metabolic level is not necessarily maintained up to the level of plant growth rate. A more likely adaptive significance of the C<sub>4</sub> mechanism of carbon concentration

is the maintenance of growth in water-deficient conditions when open stomata and conventional gaseous exchange may result in water stress. In this context Laetsch (A. Rev. Plant Physiol., 25, 27; 1974) has pointed out that the C4 mechanism is commonest in plants subjected to intermittent aridity whereas the somewhat related process, crassulacean acid metabolism, is associated mainly with plants experiencing permanently dry conditions. As far as productivity is concerned the ecologist and crop physiologist must evidently apply caution before extrapolating from biochemical data.

Peter D. Moore

# **Inertial frames** in relativity

The results of Bishop and Landsberg (page 459) have clarified the rather tangled skein of exposition of relativity theory. They have relevance not only for discussions of the principle of equivalence, which is their concern, but also for the exposition of special relativity which Dingle has attacked on so many occasions. Dingle's attacks have shown the exposition of special relativity to be inadequate, though it is doubtful whether he has shown any deficiencies in the theory.

The key to the whole matter lies in the notion of an inertial frame. Such a frame of reference for space and time measurement occurs both in Newtonian mechanics and in special relativity, and it is assumed in most treatments that the occurrence is the same in each case. Such a view underlies Einstein's heuristic argument by which he managed, as long ago as 1911, to derive from the Doppler shift the gravitational red shift of spectral lines. The essential step here is to transform to a freely falling frame of reference so that the acceleration due to gravity is abolished, by the principle of equivalence, of bodies at a point having the same acceleration. The acceleration in the original frame produces a velocity in the new one, and consequently the Doppler shift. The conclusion is that clocks which were originally synchronised are slower deep in a gravitational field so that the geometry of space-time is modified. Though this argument is only heuristic, it was important for suggesting the principles of general relativity to Einstein, but, as Bishop and Landsberg point out, it is obvious nonsense, since it is entirely carried out in Newtonian physics and therefore a transformation of time is evidently in contradiction to the absolute time concept.

The difficulty lies in the fact that both frames of reference cannot be inertial. Bishop and Landsberg claim to have a theory that gives time stretching in Newtonian mechanics, and gives the Einstein result to the first order in special relativity. Physically, this is not inconsistent with Price's result (Am. J. Phys., 42, 336) earlier this year with the Einstein formula holding exactly in special relativity. The discrepancy arises only from slightly different definitions of acceleration.

The letter from Bishop and Landsberg, however, leaves unconsidered just what is the intrinsic difference between the two theories, and this seems to lie in the different concepts of inertial frames. In special relativity, such a frame is one in which Newton's laws and Maxwell's equations have their usual form, and it is then a text-book exercise to show that any two such frames are connected by the Lorentz transformation, and that the frames are in uniform relative motion. Essential for this derivation is that we have an inertial frame to start with, so in special relativity there is a six-fold infinity of preferred reference frames. The situation in Newtonian mechanics is completely different: the inertial frame is defined very simply by the form of Newton's laws. Then every frame in uniform

relative motion is also inertial and the transformation between them is then a Newtonian approximation to a Lorentz transformation. But there is no preferred set of frames in the absolute sense of special relativity to start with, because, once gravitational fields are allowed, there is no precise set of forces and any frame of reference can be considered inertial so long as we are prepared to introduce sufficiently strange gravitational fields. The only sense in which the six-fold infinity of frames can be said to be preferred is that of convenience.

The basis of Dingle's long-standing arguments with the relativists seems to be his insistence on the arbitrary nature of inertial frames. Neither his antagonists nor he himself have noticed that this arbitrariness is explicitly denied. The current discussion makes all this a little clearer.

C. W. KILMISTER

# Element synthesis and isotope anomalies in the early Solar System

from Grenville Turner

THE chemical elements which make up the Solar System were synthesised by various nuclear processes which occur at different stages in the life cycle of stars. Much of the experimental evidence which verifies in detail the operation of these processes is to be found in elemental and isotopic abundances within the Earth, meteorites and recently, the Moon. Many types of process are known to occur and there are different stellar environments appropriate to each; yet significantly, isotopic abundances are, with very few exceptions, within error uniform throughout those parts of the Solar System so far sampled. This observation implies that the material which came together to form the Solar System was at a very early stage homogenised to a very high degree.

Most of the exceptions to this rule (referred to as isotope anomalies) can be understood in terms of the radio-active decay of some long lived precursor isotope and provide the means of determining such things as the age of the Solar System and the duration of some of the element-building processes. Other exceptions in meteorites and lunar samples can be understood in terms of currently operating nuclear processes induced by cosmic rays. These processes are well understood.

Recently two isotope anomalies have been discovered in the very primitive Allende meteorite. Neither anomaly is currently understood but they may result from nuclear processes operating immediately prior to or during Solar System formation. The first of these anomalies to be found (Clayton et al., Science, 182, 485; 1973) occurs in oxygen in A1-rich inclusions present in Allende. Correlated variations  $(^{18}O/^{16}O)$  and  $(^{17}O/^{16}O)$  ratios can be most easily understood in terms of the presence of variable amounts of the single isotope 16O. Whether this is the correct interpretation or not it seems difficult to escape the conclusion that one is seeing in Allende the results of the incomplete mixing of the products of nuclear synthesis.

The second recent observation concerns the isotope <sup>26</sup>Mg. <sup>26</sup>Mg is the daughter product of <sup>26</sup>Al, an isotope which has aroused much speculation as a possible heat source during the early history of the Solar System. The need for a heat source capable of heating planetary objects on a rapid time scale arises from a number of arguments. Chemical arguments based on the presence in meteorites and the Earth of volatile elements suggest that solid material originally condensed at relatively low temperatures to form the Solar System. In contrast there is much evidence that a number of processes requiring high temperatures occurred within the first hundred million years of Solar System history; for example, the melting of iron to form iron meteorites, and magmatic differentiation of rocks on the Moon and in certain classes of stone meteorites. The heat source responsible is currently a matter for speculation, 26Al being only one of a number of possibilities.

Stimulated partly by the search for a heat source several attempts have been made to search for the 26Mg anomalies which would result from in situ decay of 26Al. Two factors make the search a difficult one. First of all <sup>26</sup>Al has a geologically short half life, 7.4×105 yr, and so the search must be confined to the most primitive of meteorites to have any chance of succeeding. Second, Mg is itself an abundant element and the anticipated anomalies would be undetectable in all minerals but those most poor in Mg and rich in Al. The search for 26Mg anomalies has had a chequered career and ar early claim to have detected them (Clark et al., J. geophys. Res., 75, 448; 1970) was later shown by an exhaustive series of measurements to have been incorrect (Schramm et al., Earth. planet. Sci. Lett., 10, 44; 1970).

A recent issue of *Nature* contains one of the latest additions to the saga. Gray and Compston (*Nature*, **251**, 495; 1974) have continued the search in the Al-rich inclusions of Allende, following the observation of the <sup>16</sup>O anomalies and encouraged by Sr isotope data

which indicate that these inclusions are the most primitive material known to man (Gray et al., Icarus, 20, 213; 1973). After a series of measurements, on the meteorite and three inclusions, they are able to report a small but significant anomaly for one of the inclusions which would correspond to an 0.4% excess of 26Mg. The (Al/Mg) ratios of the other two inclusions are less favourable and an anomaly, if present at an equivalent level, would be barely detectable. Gray and Compston conclude that if the anomaly is the result of in situ 26Al decay then the implied initial concentration of 26Al would represent a significant early Solar System heat source.

A second group of research workers have been searching for Mg anomalies in Allende and their results have also been published recently (Lee and Papanastassiou, Geophys. Res. Lett., 1, 227; 1974). This work, coming from the laboratory which painstakingly laid to rest the early claims of <sup>28</sup>Mg anomalies, indicates that, in Allende at least, anomalies are present. A most significant finding is however that in one of the samples the anomaly is negative—which appears to rule out in situ decay of <sup>28</sup>Al as the cause of the anomalies.

Lee and Papanastassiou have performed 39 analyses on 21 distinct samples from Allende and found anomalies in nine of the samples. The anomalies are found in the Al-rich inclusions and expressed in terms of the (26Mg/ <sup>24</sup>Mg) ratio range from -0.17% to +0.30% with typical uncertainties of around 0.02%. The shifts are much smaller than those found in oxygen, which range up to 5%. Moreover there seems to be no direct correlation with the oxygen anomalies in that the samples with large oxygen anomalies show no magnesium anomaly. There is also no correlation with (Al/Mg) ratio and this in addition to the negative <sup>26</sup>Mg anomaly referred to above argues against in situ decay of 26Al. The authors suggest a number of possible nucleosynthetic processes which could lead to enrichment or depletion of various Mg isotopes but argue that the absence of a correlation with the oxygen anomalies hinders the identification of a single process as the cause.

In summary there is now a growing body of evidence for the presence in meteorites of the products of nucleosynthesis which have not been thoroughly mixed. To the O and Mg anomalies should be added two as yet unexplained anomalies in the inert gases Xe and Ne (Black. Geochim. cosmochim. Acta., 36, 377; 1972). The explanation of these effects is a challenge which, if met, will add greatly to our understanding both of the processes of nucleosynthesis and of the early evolution of the Solar System.

### A promotory effect of inflammation on tumour initiation

from A. J. S. Davies

To its devotees tumour immunology is an absorbing pastime. To them the facts that T lymphocytes and B lymphocytes and macrophages can all in their different ways be cytotoxic, at least in vitro, are mutually compatible. The existence of anti-tumour antibodies that can be thought of as cytotoxic or enhancing or blocking is regarded with joy. The possibility that antibody-tumour-antigen complexes or tumour antigen alone can reduce the aggressive anti-tumour effects of some of the various kinds of hostile lymphocytes becomes part of the grand design. On this euphoric wave of complexity the tumour immunologists are carried as they invent even more and bizarre ways of immunotherapy. Their methodology comes increasingly to resemble some of the early attempts to control bacterial diseases by methods that retrospectively were seen to be nonspecific and unreliable.

This is not to say that the tumour immunologists are wrong all the way along the line. There may really be an immunological surveillance mechanism which eradicates most tumours before they become a nuisance. When this mechanism has failed there may be tumour-specific immune responses in all instances which can be manipulated for the benefit of the patient. But the greater the economic pressure on scientists engaged in this biomedical research the more positive will be the assertions, not necessarily justified, that a particular theory is correct. A recent paper in the British Journal of Cancer by van den Brenk et al. (30, 246-260; 1974) blows a breath of fresh air into the arena.

Van den Brenk and his colleagues have worked on the effects of various inflammatory and anti-inflammatory agents on the survival and growth of clones of injected foreign tumour cells in rats. For example, they found that injection of cellulose sulphate ten minutes before intravenous injection of the Walker-256 adenocarcinoma caused substantial increases in the number of tumour cells starting to grow in the lungs and as a consequence a marked increase in tumour weight. This was even true in immune mice though immunisation always had some protective effect. From other experiments cellulose sulphate is known to be a powerful inducer of inflammation by some mechanism that is not altogether clear. Cellulose sulphate is also an anticoagulant and can be fibrinolytic.

In spite of the complexity of its effects the authors of the experiments feel and present additional evidence that the inflammatory properties of cellulose sulphate are those responsible for its augmentary effect on tumour seeding and thus tumour growth. In other experiments injection of a lung homogenate is shown to have a similar augmentary effect by means which are thought to be pharmacologically the same as those involved in the effect of cellulose sulphate. Direct effects of either lung homogenate or cellulose sulphate on tumour growth seem to be ruled out in control experiments. Experiments were performed in which tumour and inflammatory agents were injected in the rats' forepaws. Again cellulose sulphate caused some enhancement of tumour growth.

It would be nice to see these experiments repeated with syngeneic tumour implants. But there are many interesting corollaries of the present findings which should be read about in the original. One of the specific points made by the authors is worth underlining here. They say that a situation can arise wherein the survival and growth of tumour cells are inhibited by immunity but stimulated by inflammation resulting from the destruction of participating host and tumour cells. This provides food for thought for tumour immunotherapist and radiotherapist alike especially as Stjernswärd now claims to have evidence (Lancet, ii, 1285; 1974) for decreased survival related to postoperative radiation in early operable breast cancer. Van den Brenk and his associates have shown moreover that injection of an antiinflammatory steroid could reduce the augmentary effect of cellulose sulphate on the seeding of tumour cells. The old fashioned view that tumours should be thought of in the light of the naturenurture paradigm is perhaps worth reconsideration particularly if damaged cells can nurture (albeit indirectly) their intact tissue-mates.

# Superfluid <sup>3</sup>He at zero pressure

from P. V. E. McClintock

LIQUID <sup>3</sup>He has been cooled to temperatures below 0.7 mK in a nuclear demagnetisation cryostat and, under its own saturated vapour pressure, it was found to undergo a superfluid transition at 0.93 mK. The experiment, which was performed at the Helsinki University of Technology by Ahonen, Haikala, Krusius and Lounasmaa, is described in a recent issue of *Physical Review Letters* (33, 628; 1974).

Since the discovery in 1972 that

liquid <sup>3</sup>He under a pressure of 24 atmospheres undergoes two separate transitions at temperatures of about 2.7 and 2.2 K, intensive efforts, both theoretical and experimental, have been made towards gaining an understanding of the two new phases of the liquid. The experiments have, until now, all employed one of two cooling methods: either compressional solidification of 3He, known as Pomeranchuk cooling, which restricts experiments to the solidification pressure of 34 atmospheres; or adiabatic demagnetisation of cerium magnesium nitrate (CMN), which can be carried out at any pressure. Using these techniques, a remarkable amount of information concerning the nature of the new phases has been gained from a variety of experiments including, in particular, nuclear magnetic resonance (NMR) measurements. It has been shown that both the higher temperature so-called A phase and the lower temperature B phase exhibit superfluid properties; and that, as the pressure is reduced with zero applied magnetic field, the higher temperature transition moves to lower temperatures, while the lower temperature one moves to higher temperatures, with the two transitions meeting in a tricritical point at about 20 atmospheres. Below 20 atmospheres only the B phase is stable, and the transition was found to move to lower temperatures with falling pressure. It was not feasible by demagnetising CMN to follow the transition below about 15 atmospheres and 2.2 mK, so that is was impossible to say whether or not the transition would ever reach zero pressure: it seemed quite credible that 3He might not be superfluid under its saturated vapour pressure, but would always require the application of external pressure to undergo the superfluid transition.

The ultimate low temperature attainable with CMN is related to the spontaneous ordering of the electron spins on the cerium ions as a result of their mutual magnetic interaction. To reach temperatures significantly below 2 mK an assembly whose magnetic elements interact less strongly is therefore required, and the Helsinki group chose to demagnetise copper nuclei in order to achieve their cooling effect. In essence, the technique is simple. When a large magnetic field is applied at a starting temperature of less than 20 mK, the copper nuclei tend to align themselves, and heat is liberated and is removed by means of a dilution refrigerator. The thermal link to the refrigerator is then broken and the magnetic field very slowly reduced to zero, resulting in cooling as the nuclei disorientate themselves again.

In practice, great care has to be taken in minimising heating due to the eddy currents which are liable to be induced in the copper as the field is run down; and possible heat leaks, whether through conduction, radiation or mechanical vibration, have to be forseen and eliminated by suitable design as far as is humanly possible. To avoid the eddy current heating the Helsinki group used a very large bundle of separate high purity 0.1 mm diameter copper wires, insulated from each other along most of their length. The top ends of the wires, well away from the demagnetising field, were all welded together into a piece of copper, which in turn was welded to the copper chamber which held the 3He.

Earlier calculations had suggested that extreme difficulties might be encountered in achieving good thermal contact between the copper and the liquid 3He, with a thermal boundary resistance varying as T<sup>-3</sup> and the probability that nuclear demagnetisation could not be used to cool 3He below 2 mK. For this reason the cell was designed in such a way as to present as large as possible a surface area of copper to the liquid: most of the volume was filled with a sponge of sintered copper powder, so that the 6 cm3 of 3He was in contact with about 30 m<sup>2</sup> of copper surface. In fact, the authors found that the problem was much less severe than had been anticipated, probably owing to thermal transfer through an unforseen direct interaction between the nuclear magnetic moments of the 3He atoms and the electronic magnetic moments of manganese impurities in the copper, resulting in a boundary resistance which varied only as  $T^{-1}$ .

Demagnetising from a starting temperature of 17 mK over a period of 3.5 h, a final temperature for the <sup>3</sup>He of just below 0.7 mK was achieved. A measure of the quality of the experimental design is given by the fact that it then took 20 h to warm up to 1.0 mK.

By performing experiments at various pressures, and detecting the phase transitions by means of characteristic changes in the NMR signals, the authors traced out the phase diagram, and were able to follow the superfluid transition right down to zero applied pressure at a corresponding temperature of 0.93 mK. This means that, at least in principle, it should now be possible to do experiments involving the free surface of the liquid.

This impressive cryogenic achievement by the Helsinki group means that, for the moment, they enjoy a clear lead over all competitors. It will be interesting to see how they will exploit their advantage, and what illuminating experiments they will perform to eluci-

date the nature of the new superfluids, particularly that of the relatively unknown B phase, now that they have developed the necessary technology.

# Receptors for neurotransmitters

from Key Dismukes

THE mechanisms by which a neurotransmitter, interacting with postsynaptic receptors, induces characteristic neural response remain shrouded in mystery. In recent years much has been learned about hormone receptors in various tissues by probing their interactions with radiolabelled ligands, compounds known to be bound by the receptor with great affinity and selectivity. Synaptic receptors in the brain present a far more difficult problem because of the heterogeneity of neurones, relatively low concentrations of receptors, and thir occlusion within the synapse. Nevertheless, there is great impetus to study these receptors, both to elucidate basic neural mechanisms and because many important psychoactive drugs operate through these sites.

The crucial step in biochemically. characterising a receptor is to demonstrate that a ligand (often an analogue of the endogenous neurotransmitter) incubated with tissue homogenate selectively binds the receptor in question. Early reports of isolation of acetylcholine receptors from brain have been discounted because the binding was apparently nonspecific, failing to parallel known pharmacological properties of the receptor. Extremely low concentrations of ligand must be used because the limited number of true receptors is soon completely occupied, whereas nonspecific tissue binding increases indefinitely with ligand concentration.

Pharmacologists have described two kinds of acetylcholine receptor: nicotinic and muscarinic. The nicotinic receptor of the electric organ of electric eels was the first to be identified biochemically, and has been investigated extensively by several groups. Although the nicotinic receptor in mammalian brain tissue has resisted definitive biochemical demonstration, several groups have successfully studied muscarinic receptor properties in mam-•malian brain and peripheral tissues. As long ago as 1965, Paton and Rang detected binding of radioactive atropine, the classic muscarinic antagonist, to the guinea pig intestine (Proc. R. Soc., B163, 1; 1965). But the low specific radioactivity of the atropine then available precluded extensive biochemical characterisation.

Recently, Yamamura and Snyder

have examined the binding of a potent muscarinic antagonist, 3-quinuclidinyl benzilate (QNB), using techniques developed for studying the opiate receptor (Proc. natn. Acad. Sci. U.S.A., 71, 1725; 1974). Binding of 3H-QNB was found to be almost completely blocked by excess cold QNB, indicating that the interaction was saturable, and thus specific. Muscarinic antagonists and agonists displaced specific 3H-QNB binding, but nicotinic and non-cholinergic agents showed no affinity for ONB binding sites. These experiments indicated a concentration of muscarinic binding sites similar to that of the nicotinic receptor of the electric organ of the electric eel. The electric organ has been presumed to be extremely densely innervated, but apparently is no more so than the brain is with muscarinic receptors—which is notable, considering that only a small portion of the brain's synapses are likely to be muscarinic.

Burgen, Hiley, and Young have found closely similar features of muscarinic cholinergic receptor binding in rat brain by using <sup>3</sup>H-propylbenzilycholine, an antagonist related to nitrogen mustard which binds irreversibly to the receptor (*Br. J. Pharmac.*, **51**, 279; 1974). Similarly, Soudijn, Wijngaarder, and Ariens have had success with an atropine-like agent, dexetimide (*Eur. J. Pharmac.* **24**, 43; 1973)

Rigorous evidence that QNB binds specifically to acetylcholine receptors was obtained by comparing the pharmacological potency of a number of cholinergic agents with their ability to inhibit 3H-QNB binding in guinea pig ileum. Acetylcholine is known to produce contractions of the guinea pig intestine through a muscarinic receptor. For each muscarinic agent a close parallel was found between the concentration necessary to block 3H-QNB binding 50% and the concentration producing half-maximal response of the intestinal strip. Nicotinic agents and non-cholinergic drugs which do not stimulate the ileum did not alter 3H-QNB binding.

The team of Yamamura, Kuhar and Snyder is now using 3H-QNB binding to examine the distribution of muscarinic receptors in brain. Binding was examined after destruction of septal cholinergic tracts to the hippocampus (Brain Res., 78, 320; 1974). Although cholinesterase activity was drastically reduced by this treatment, there was no alteration of 3H-QNB binding, suggesting exclusively post-synaptic localisation of these receptors. A detailed regional map of receptor concentration has been obtained in monkey brain (Brain Res., 66, 541; 1974). Because of its extremely high affinity, 3H-QNB can selectively label the brain's muscarinic

receptors even when administered in vivo. After in vivo labelling of receptors, it was found possible to perfuse the brain to preserve microscopic structural integrity, thus permitting direct visualisation of receptors by autoradiography (Brain Res., 80, 170; 1974)

Using procedures similar to those of the acetylcholine work, Young and Snyder have studied the binding of <sup>3</sup>H-strychnine to synaptic membrane fractions from spinal chord and brain stem (Proc. natn Acad. Sci. U.S.A., 70, 2832; 1973). Strychnine is known to be a potent and specific antagonist of glycine, a major inhibitory neurotransmitter. The binding of strychnine seems to be associated with the synaptic glycine receptor, since the ability of glycine and various other amino acids to inhibit 3H-strychnine binding closely parallels their glycine-like neurophysiological activity. Furthermore, the distribution of <sup>3</sup>H-strychnine binding in the brain closely resembles the distribution of endogenous glycine, neuronal sensitivity to and nerve-ending uptake of glycine.

Interestingly, Young and Snyder find that glycine and strychnine seem to bind to interacting, but separate portions of the receptor (Molec. Pharmac. 10, 790; 1974). Glycine displaces 3H-strychnine in a cooperative manner, and its binding can be blocked by various reagents which do not interfere with the binding of strychnine. The inhibitory postsynaptic potential (IPSP) caused by glycine can be reversed by iontophoretic injection of various anions, apparently on the basis of their ability to traverse the membrane's ionic gate for chloride. A close correlation was discovered between the ability of a series of anions to reverse the IPSP and their displacement of bound 3Hstrychnine. This suggests that the binding of strychnine is closely associated with the chloride conductance gate triggered by the glycine receptor. This discovery may be a valuable tool to elucidate the mechanisms by which receptor activation alters membrane conductance.

One of the most intriguing questions of receptor theory is the difference between agonists and antagonists. Why does one type of agent binding the receptor stimulate the characteristic cellular response and another type, binding equally well, produce no effect? Several theories have been proposed, but pharmacological data are not sufficient to distinguish any one. These biochemical binding techniques, being fervidly developed in several laboratones, allow scrutiny of receptor interactions at a level which may answer this and other basic pharmacological questions.

### Gallium nitride, a valuable semiconductor

from A. G. Holmes-Siedle

Semiconducting compounds of elements in groups III and V of the periodic table, such as gallium arsenide (GaAs) could be called the second generation of useful semiconductors. They are isoelectronic with silicon but generally adopt the zinc-blende rather than the diamond crystal form. This leads to a considerable difference in the band structure and to the manyvalley feature which permits the very useful Gunn oscillations in GaAs. The array of possible defect centres is intrinsically more diverse than that for silicon because either of the two types of atom present can be replaced in the lattice. This has not prevented the development of devices which employ the radiation emanating from the defect centres during electron-hole recombination. (These devices, light-emitting diodes and injection lasers, have greatly improved numerical displays and may vet revolutionise communications.) By mixing the compounds of gallium, aluminium, arsenic and phosphorous, layered junction structures can be made which emit light of wavelengths varying from 0.5  $\mu$ m (2.4 eV, green) to  $0.9 \mu m$  (1.4 eV, infra-red). One member of the series, however, gallium nitride (GaN) is not so easily prepared and does not mix successfully with the other members; this is a pity since it is a material with great potential.

Of all the III-V compounds, GaN has the widest forbidden band gap (about 3.5 eV) and can be induced to luminesce in the blue, violet or ultraviolet regions (the ultraviolet starts at 0.4  $\mu$ m). Also, since the band-edge absorption occurs in the ultraviolet (0.35  $\mu$ m), pure crystals of GaN are colourless and transparent. In spite of the wide band gap, crysals as grown may often have resistivities as low as silicon and also have fairly high carrier mobilities; if it could be made, the intrinsic or "i" material would be of very high resistivity indeed.

Clearly this is an unusual and potentially useful semiconductor although preparative problems have prevented its use in electronic devices up to now. Not only is it difficult to control the impurities which give high electron concentrations (n-type material); it is equally difficult to find and introduce impurities which produce p-type material. Thus, no good method of forming a p-n junction structure (an integral part of the conventional light emitter) has been found. But there are signs that other practical methods may be found to produce a luminescing

film, for example the generation of avalanche currents by pulsed fields (Pankove and Norris, RCA Rev., 33, 377; 1972) and there are now signs that GaN may be nearly ready for use in device technology. It may thus be worth while for physicists and chemists to survey some of the known facts about the physics of GaN, aided by a concise review by F. P. Kesamanly (Soviet Physics, Semiconductors, 8, 147; 1947).

A serious drawback to incorporating GaN into the conventional layered III-V alloy laser structures is that, in contrast to all the other III-V compounds shown in Table 1, which crystallise in the zinc-blende form, GaN has the wurtzite structure. GaN was first synthesised in 1932, well before the discovery of semiconducting properties in the III-V compounds. Growth of large single crystals has, however, always proved difficult. Only recently has it been found possible to grow the desired large-area films by epitaxial growth on sapphire, using the disproportionation of gallium chloride and ammonia. This can yield films up to 240  $\mu$ m thick with an electron density of 2.1017 cm-3 and an electron mobility of  $380 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ at room temperature. It has not generally been possible to grow films of lower electron density. But there are reports that Pankove has produced layered structures in which semi-insulating GaN film (that is, electron density presumably well below 1014 cm<sup>-1</sup>) is overlaid on a strongly n-type film deposited on sapphire. This may give the essential parts of an avalanche electroluminescent diode which can possibly emit light at fairly high power.

While the semiconductor world was awaiting the development of a suitable structure for carrier generation in GaN, investigators were assessing the luminescent transitions by photoexcitation techniques, starting with Grimmeiss and co-workers in 1960 (Z. Naturforsch, 15a, 799). It was encouraging to find several strong luminescence peaks in the ultraviolet, at 3.47 eV (0.36  $\mu$ m), 3.29  $(0.38 \ \mu m)$  and 3.16  $(0.39 \ \mu m)$ . R Dingle and colleagues (J. Appl. Phys., 43, 3797; 1972) proposed that the 3.4 eV band was the result of recombination of excitons bound to neutral donors and neutral acceptors. Grimmeiss

Table 1 Comparison of properties of III-V semiconductors (300 K)

•	GaN	GaP	GaAs	GaSb	)
Band gap	3. <i>5</i>	2.24	1.43	0.67	
Electron mobility	380	110	8,500	400	
D J	InN	InP	InAs	InSb	AlSb
Band gap	<1.9	1.29	0.33	0.16	1.63
Electron mobility	<b>— 4</b> ,	600 3	3,000	78,000	200

instead attributed the 3.4 eV peak to recombination of free excitons. The doping of GaN with impurities either gives rise to new bands or enhances the triplet. Grimmeiss reports that the doping of gallium nitride with lithium, sodium, copper, silver, gold, zinc, cadmium, barium, mercury, tellurium, tin and lead gives rise to photoluminescence bands with maxima at twelve different wavelengths in the violet to yellow range; mercury produces a band with a maximum at 2.1 eV (0.59  $\mu$ m, orange). Doping with beryllium, magnesium, calcium, aluminum, and indium produces no new bands but enhances the ultraviolet emission. Soviet workers have found acceptor levels on doping with zinc, cadmium, and lithium; these levels are located 0.40, 0.69, and 0.95 eV above the top of the valence band.

Gallium nitride has been used in the construction of luminescent MIS (metal-insulator-semiconductor) diodes with a Si<sub>3</sub>N<sub>4</sub> insulator film 0.1 μm thick (Pankove and Norris, loc. cit.). The luminescence spectra of such diodes include a wide band of 2.1 eV energy (0.59  $\mu$ m, orange) and an ultraviolet band at 3.28 eV. Green-emitting MIS diodes based on zinc-doped GaN and magnesium-doped GaN have been made in this way: the latter material emitted in the violet part of the spectrum. The efficiency of these structures was 10<sup>-5</sup>. Diodes emitting yellow light and characterised by an efficiency of 4.10-4 were prepared from n-i structures with the high resistivity regions doped with zinc. This is comparable to the efficiency of gallium phosphide green light emitters. In these n-i structures, the light was produced by a pulsed voltage, of, say, 30 V, applied to a metal electrode over the i layer. The light emitted could, of course, be viewed readily through the sapphire substrate. and segmented displays have been made in this fashion. Moreover, it is understood that these devices have operated for several months without degradation in performance. Kesamanly (loc. cit.) also reports that GaN will alloy with indium nitride and that the band gap is adjustable thereby.

It thus seems that GaN is on the threshold of becoming a new tool in the hands of the semiconductor physicist. It will be more versatile than other III-V compounds both because the diodes will emit light over the whole of the visible range and because GaN is completely transparent in the visible. It will be of great interest to see what benefits this will bring to our technological repertoire and which country (the United States, United Kingdom, Soviet Union, Germany) leads the way in developing new gadgets—say a whitelight laser or solid television displayfrom this advance.

# Dilatancy without fluid flow?

from Peter J. Smith

It should be quite possible to predict earthquakes on a purely phenomenological basis with little, if any, understanding of the physical process involved. All that would be necessary would be to find a premonitory effect which is common to all earthquakes, or at least to a class of earthquakes large enough to give prediction some practical significance. In recent years, for example, it has been observed that, before some shallow events, the seismic wave velocity ratio  $V_P/V_s$  suddenly decreases and then rises again to its normal value just before the shock takes place. This phenomenon may or may not turn out to be a way of predicting shallow earthquakes on a routine basis; but whether it does or not need not depend on any insight into the cause of the velocity changes.

Nevertheless, there is some advantage in having a physical model to explain the observations. For one thing, it tends to inspire confidence; and if there is one thing likely to be required in abundance when it comes to practical prediction, it is confidence. More specifically, an understanding of the mechanisms involved may lead to the discovery of new relationships, and thus bring practical prediction closer. For example, there can be little doubt that the dilatancy model, first proposed by Nur (Bull. Seismol. Soc. Am., 62, 1217; 1972) and subsequently developed and modified by others, has done almost as much as the observations themselves to produce the new wave of optimism in prediction work. By relating the various premonitory effects such as  $V_p/V_s$ changes, resistivity variations and the migration of seismicity to a common mechanism and a common precursor time-earthquake magnitude scale, the dilatancy theory has produced the comfortable feeling that everything is beginning to fit nicely together.

But is the theory correct? Or to put the question in the more limited form that Stuart (Geophys. Res. Lett., 1, 261; 1974) now puts it: are the current dilatancy models involving fluid flow correct? Insofar as there have been no serious objections to the dilatancy-fluid diffusion mechanism, the question may seem superfluous. But as Stuart points out, the theory apparently defies Occam's razor by introducing fluids without a proven need. Moreover, there are good scientific reasons for questioning the occurrence of fluid diffusion, described by Stuart as the "requirements of ubiquitous finite permeability, existence of pore fluid, and ostensibly great distances involved for large

earthquakes".

Stuart's new model has the earthquake occurring within a relatively thin shear zone which has mechanical properties quite different from those of the crustal rocks further away. In the shear zone, the energy applied during deformation is dissipated by creep. In the surrounding crustal rocks, on the other hand, the deformation energy is stored elastically until such time as it is released in an earthquake. Assuming that the two regions are each homogeneous and in welded contact, their stresses at any time will be similar but the strains will differ significantly. To a first approximation the outer rocks will have a linear stress-strain relationship, whereas the shear zone is assumed to have a stress-strain curve possessing a maximum stress.

The crustal rocks in Stuart's model thus have properties comparable to those of the material in conventional dilatancy models; and these rocks are likewise assumed to possess cracks and fractures. The crucial difference is the non-linear behaviour in the thin shear zone. As the stress applied to the combined system increases, dilatancy will occur as the cracks open in the crustal rocks, giving rise to a decrease in  $V_p/V_s$ . But as the stress continues to increase, dilatancy in the crustal rocks will reach a maximum and then decrease as the cracks begin to close up again,  $V_P/V_S$ will correspondingly increase. The earthquake will occur when  $V_p/V_s$  is back to normal, if the energy conditions are favourable. (If the conditions are not so favourable. Stuart's model suggests that rapid creep might occur instead; in other words, the model implies that similar premonitory events herald both earthquakes and accelerated creep.)

It seems, therefore, that the  $V_{\rm P}/V_{\rm s}$  changes observed in the field may be explained quite well without involving fluid; the inflow of fluid (to raise  $V_{\rm P}/V_{\rm s}$ ) in conventional dilatancy models is replaced in Stuart's model by closing cracks. Fluids may indeed be present, but they are not necessary to the argument. Qualitatively, the two models predict similar precursory variations in  $V_{\rm P}/V_{\rm s}$ , resistivity, and so on.

But there is at least one difference which should enable the models to be distinguished. In Stuart's model the dilatancy reaches a maximum and decreases to vanishing point before the earthquake as the cracks close. In the diffusion model the dilatancy goes on increasing up to the earthquake. Moreover, because it is diffusion controlled, the dilatancy in the latter case will only decrease slowly after the shock. In time, therefore, it should be possible to test the validity of the two models by geophysical measurement.

### review article

### Retarded cores, black holes and galaxy formation

John Gribbin\*

Recent astronomical observations have given rise to suggestions that black holes may reside at the centres of many or all galaxies. The evidence remains equivocal; and in the meantime there is a theoretical variation on this theme, in which galaxies grow on retarded cores — 'white holes' — which have been present since the initial big bang.

During the past few years the concept of black holes has moved out of the specialist astronomical literature into a broader arena of discussion. This has come about chiefly because of the interest aroused by the discovery of sources of intense X-ray emission which seem, from the periodic variations of systems which are taken to be binary stars, to be too massive to exist as stable stars without producing visible optical radiation. Since no optical counterpart can be detected for these objects, it has been suggested that they have evolved from burnt out massive stars which have collapsed, now that nuclear reactions no longer provide the energy necessary to hold them up, into black holes.

The idea is certainly attractive, and it has the advantage that such a collapsed object would indeed be able to 'drive' the observed X-ray emission, through the conversion of gravitational potential energy into radiation as matter falls into the black hole. It is not yet clear, however, that it is strictly necessary to invoke black holes to explain the X-ray sources observed so far; the evidence is persuasive, but other more or less bizarre ideas (such as differentially rotating white dwarf stars, or models based on triple rather than binary systems, see ref. 1) can also be made to fit the observations.

Whether or not they prove to be the correct explanation of X-ray sources, the excitement aroused by recent work has tended to obscure the fact that black holes are not a recent idea. In fact, such objects have been contemplated by the mathematicians for many years, and whether or not stellar mass black holes exist in star systems in our Galaxy that kind of object could play an important part in the evolution of the Universe, or of individual galaxies. In many ways, the concepts involved in studying the consequences of the existence of such large mass black holes are just as exciting as the possibility that smaller black holes exist in our immediate astronomical neighbourhood; so this may be a good time to examine recent progress from these older ideas.

### Observational evidence

It seems likely that elliptical galaxies contain massive 'black holes'—objects collapsed within their Schwarzschild radii—in their nuclei (see, for example, Wolfe and Burbidge²). The principal evidence in favour of this concept is the 'missing mass' problem; the average mass of a bright elliptical is  $8\times 10^{11}~M_{\odot}$  and the mass-to-light ratio is about 70, implying that the light from these ellipticals can be explained in terms of the emission from stars which make up only some 25% of the mass. There is no similar mass-to-light problem with spirals, but the data are insufficient to rule out the possibility that spirals (including our own Galaxy, see ref. 3), also contain black holes.

But if black holes do exist at the centres of galaxies, it is

something of a problem to account for the evolution of them in terms of gravitational collapse. Wolfe and Burbidge mention in their paper the possibility that such central objects might be related to the 'retarded cores' discussed by Novikov<sup>4</sup> and by Ne'eman<sup>5</sup>, and by others since 1965. A straightforward calculation in the Newtonian approximation shows that the constraints imposed on the nature of elliptical galaxies by assuming that they form on such cores are in complete accord with the limits imposed on the masses of the central black holes from a study of the structure of elliptical galaxies.

### Limits on the size

Following Wolfe and Burbidge<sup>2</sup>, I take the energy generation 'requirement' of an elliptical galaxy to be  $10^{81}$  erg, allowing the galaxy to become a strong radio source. This is equivalent in mass terms to  $10^7 \, M_{\odot}$ , and since there is evidence that recurrent outbursts can occur in radio galaxies, a total of some  $10^9 \, M_{\odot}$  seems to be required to power the observed phenomena. For such a mass, the Schwarzschild radius  $(R_s)$  is about  $10^{-4}$  pc; for a mass of  $10^{11} \, M_{\odot} \, R_s$  is about  $10^{-2}$  pc. There seems little doubt that the energy which drives radio sources must be gravitational in origin, and according to Wolfe and Burbidge "on the basis of conventional theory, it must be argued that ellipticals which have given rise to radio sources contain black holes", because of the requirement that large masses must now be concentrated within regions a few light years across in order to explain the energy production.

From studies of the velocity dispersion in the inner parts of some elliptical galaxies Wolfe and Burbidge are able to set limits on the size of any central black hole. I shall assume that the required mass is not present in the form of many smaller black holes, although this possibility cannot be ruled out by present data. It then turns out that the mass required to explain the observed distributions must in general be less than some  $10^{10} M_{\odot}$ , and that for systems which are relaxed the central mass must be less than  $10^9 M_{\odot}$  (ref. 2).

### Possible size of retarded cores

The concept of retarded cores of expansion arises naturally if we consider time reversal of the present expanding Universe. To an observer A in a region which is less dense than the region inhabited by another observer B there must come a time when observer B disappears behind the event horizon of a black hole as his region of space contracts within the value of  $R_s$  appropriate for the density it has.

\*By considering the time reversal of this situation, it seems reasonable to argue that in an expanding universe which is not of uniform density observers in regions of less than average density will see regions of greater density expanding away from their Schwarzschild radii. Such relatively dense regions would therefore appear as 'white holes', and their expansion would presumably follow essentially the same rules as the

<sup>\*</sup>Nature, London

expansion of the whole Universe. It is postulated<sup>4,5</sup> that the expansion of such cores is delayed in some way, hence their description as 'retarded cores'. Eardley<sup>6</sup> has suggested that because of accretion any white hole existing in the Universe would rapidly be turned into a black hole, because of accretion from its surroundings.

This is more or less what one would expect from commonsense arguments, but the Universe may not be run in a 'commonsense' way. Since the whole concept depends to some extent on tinkering with the boundary conditions of the big bang, it is not unreasonable to argue (P. C. W. Davies, personal communication) that the Universe adjusts itself in some way to counteract the tendency of white holes to extinguish themselves by accretion. That argument is not entirely satisfactory. But the case against white holes is not yet proven, and in what follows the existence of either black or white holes will suffice to 'seed' galaxy formation. Even if an initial white hole or delayed core is converted into a black hole, its mass will still act on outside material in exactly the same way.

It could be argued that there is no evidence that the Universe was ever in a suitable state of inhomogeneity to give rise to such cores; but then, there is no evidence that it was not, and it also seems (see Misner, Thorne and Wheeler?) that an initially random 'mixmaster' type of universe would rapidly evolve into a state very like an Einstein-de Sitter universe with some inhomogeneity. What happens to the region of the universe near to such a retarded core as the universe expands? Locally, gas will be restrained from the general expansion by the gravity of the core, and if stars form in the resulting gas cloud we may expect a galaxy to be produced.

There seems no need to look further than the Newtonian analogy of the Einstein-de Sitter universe for a first model to describe the behaviour of the cloud around the retarded core. For a homogeneous cloud of mass M we have

$$r^2 = 2GM/r$$

Ideally, the calculation should be carried through with some mass—the retarded core—kept within some confine (presumably its Schwarzschild radius) at the centre of the cloud from the start of expansion. But that involves a full mathematical treatment, and a simpler calculation in the Newtonian approximation may suffice to indicate the broad outlines of what is going on. The trick is to allow a mass  $\mu$  to appear at the origin of r at a time when  $r = r_0$ . As it stands, that assumption can be as accurate as we wish, since we have not yet specified either  $\mu$  or  $r_0$ . And if such a mass does appear then subsequent expansion will obey the equation

$$\dot{r}^2 = [2G(M+\mu)/r] - [2G\mu/r_0]$$

so that the cloud expands to a maximum radius given by

$$r_{\text{max}} = (1 + M/\mu)r_0 \sim (M/\mu)r_0 \text{ if } M \gg \mu$$

and then falls back. Since M is the mass interior to r, the outer regions expand to greater maximum radius than the inner regions.

In the Einstein-de Sitter cosmology with spherical symmetry we have

$$ds^2 = d\tau^2 - S^2(\tau)(dR^2 + R^2 d\Omega^2)$$

where

$$S \propto \tau^{2/8}$$
 and  $c = 1$ 

Transforming to locally flat space-time (for an observer at R=0) gives  $\mathrm{d} s^2 = \mathrm{d} t^2 - \mathrm{d} r^2 - r^2 \mathrm{d} \Omega^2 + (\mathrm{local} \ \mathrm{gravitational} \ \mathrm{effects})$  and the local gravitational effects may be included as a fourth order term such that

$$ds^{2} = dt^{2} (1 - 2GM/r) - dr^{2} - r^{2} d\Omega^{2}$$
 (1)

where M is the mass interior to r, proportional to  $r^3$  for uniform density. Equation (1) is the Newtonian result to first order, and the effect of a mass  $\mu$  at r=0 may be included by writing

$$ds^2 = dt^2[1-2G(M+\mu)/r] - dr^2 - r^2d\Omega^2$$

where M is now the mass of the cloud interior to r and not the total mass (cloud + core) interior to r.

It is possible to represent the solution of the local gravitational problem by a power series in the dimensionless parameter  $2G(M+\mu)/(rc^2)$  which is small for a local problem. The first term in the series is the Newtonian solution for the effect of  $\mu$  and may be used to good approximation provided that the second term in  $(GM/rc^2)$  is smaller than the first order term involving  $\mu$ , that is, if

$$2G\mu/rc^2\gg [2GM/rc^2]^2$$

This must hold for all r, including  $r = r_0$ , so

$$r_0\gg (M/\mu)2GM/c^2$$

if the problem is capable of solution by the Newtonian approximation. This corresponds to

$$r_{\text{m a x}} \gg (M/\mu)^2 2GM/c^2$$
 (2)

The argument here comes close to being circular, but seems fairly plausible. What we have found is that if the Newtonian approximation is valid, then the maximum radius reached by the expanding cloud with a retarded core at its centre satisfied inequality (2). Now, it is no secret that the whole purpose of this calculation is to come up with a mathematical description of something which looks like a galaxy. So we can put a typical galactic radius, say  $3\times10^{22}$  cm, into inequality (2) as the value of  $r_{\rm max}$ . If that is done, the inequality holds with a central mass  $\mu$  of  $10^9 M_{\odot}$  acting to regard the expansion of a cloud of mass  $M=10^{12}~M_{\odot}$ . With those figures, we can also set a limit of  $3\times10^{19}$  cm on  $r_0$ .

Putting all these figures together, we can say that for such a mass  $(10^9 M_{\odot})$  the value of  $r_0$  is much greater than the appropriate value of  $r_s$ . And, most important of all, we do indeed find that for galaxy-like objects the assumption that  $\mu \ll M$  is plausible.

All this does not, of course, prove that there must be collapsed objects at the centres of galaxies. But it suggests that the idea is not unreasonable. That is the best that can be hoped for for many astronomical ideas. The result is, however, suggestive in the light of the limit set on any central mass by Wolfe and Burbidge.

### Nature of the resulting galaxy

If we accept this result at face value it is simple to determine the optical appearance of the resulting galaxy. By considering shells of thickness dr at distances r from the centre of the gas cloud, we have a mean density at r given by

$$\rho_r = (\text{constant} \times r^{-2/3} \, dr) / (r^2 \, dr) \propto r^{-8/3}$$
 (3)

simply from dividing the mass of the shell by its volume. If stars form throughout the cloud we may take relation (3) as indicative of the number density of stars at r, and if all stars have the same luminosity function then the emissivity per unit volume at r is simply

$$E_r \propto r^{-8/3}$$

so that the intensity distribution measured by an outside observer will be

$$I_r = r E_r \propto r^{-5/8}$$

This is assuming an initially spherical distribution; but the resulting power law is in striking agreement with observations of elliptical galaxies and rather better than Hubble's well known empirical inverse square law.

Deviations from the spherically symmetric expansion may be considered by imposing a rate of strain tensor and a rotation of the cloud about the central mass. Hoyle and Narlikar8 have considered similar problems within the rather different framework of one of the C-field variants on the steady state model. They find that in such a situation the galaxy formed will be ellipsoidal with unequal principal axes, so that we would have something very similar to the elliptical galaxies observed today.

There remains the problem of spiral and irregular galaxies, and I do not wish to postulate any detailed model to account for their formation here (but see ref. 9). Evidence which suggested a two-component structure to our own Galaxy might, however, be taken as a hint that the spiral structure is a later appendage which has grown on to a retarded core type of central galaxy; and since rotation is only a second order effect which ought to be rapidly damped in the kind of expanding cloud envisaged, any evidence that elliptical galaxies are in general rotating would make the model outlined above

It is, however, striking that Wolfe and Burbidge say that any central black hole must be of mass less than  $10^9 M_{\odot}$  to

account for the structure of observed ellipticals, that the retarded cores hypothesis leads to exactly the same limit and that just this mass is required for the energy generation mechanism in radio galaxies. The model removes problems of how the collapsed objects formed, and the notion of such retarded cores as 'white holes' at the centre of galaxies encourages obvious speculations about the processes in QSOs, Seyferts and the like where matter seems to be pouring out into the Universe from local regions of space.

Even if it is necessary to adjust the boundary conditions to accommodate the existence of white rather than black holes at the centres of galaxies, that would be no worse than other ideas which have been put forward and which explain the energy produced in such objects in terms of new laws of physics and even the creation of matter.

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# articles

### Towards gamma-ray lasers

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We suggest that two types of  $\gamma$ -ray laser are possible: a gently pumped variety utilising isomer separation and the Mössbauer effect, and a vigorously pumped kind using fast neutron excitation of compressed matter.

In this article we will discuss the operation of  $\gamma$ -ray lasers, namely devices which produce coherent, monochromatic, unidirectional electromagnetic radiation from de-excitation of excited nuclear levels. We will describe the necessary conditions for  $\gamma$ -ray laser operation, and consider some possible means of satisfying them.

The lifetimes for spontaneous γ-ray emission from excited nuclei range from  $10^{-17}$  to  $10^{-6}$  s if  $\Delta J$ , the angular momentum change in the transition, is small (≤2). For low energy transitions with large angular momentum changes, the lifetime can be much longer. For example, for  $\Delta J = 4$  and energy change ~ 100 keV, the radiative lifetime can be several years<sup>1</sup>. The existence of long lived excited nuclear states is intriguing from the point of view of achieving γ-ray laser action, because relatively modest pumping powers would be needed to produce population inversions. The opportunity of exploiting the multi-MeV binding energies of nucleons in nuclei to pump long lived

transitions between even high lying nuclear levels with the thermal neutron fluxes and charged particle accelerator beams is also notable. Moreover, the extraordinarily long life of transitions with large  $\Delta J$  makes it potentially possible to create nuclear population inversion through atom-by-atom physical segregation of the excited isomeric state from predecessor nuclei and other states of the same nucleus<sup>2-4</sup>. To take such advantage of long lived nuclear excited states, however, the spectral width  $\Delta v$  of the  $\gamma$ -ray emission line must be sufficiently small. The threshold inversion density for producing  $\gamma$ -ray laser action is proportional to  $\Delta v \tau_{spon}$ , where  $\tau_{spon}$  is the spontaneous radiative lifetime of the excited state. Thus a large value of  $\tau_{spon}$ must be compensated by a small value of  $\Delta v$ , in order that the density of the laser medium be that of a solid or less. If sufficiently small values of  $\Delta v$  cannot be achieved, then shorter lived excited levels, together with a density of the  $\gamma$ -ray laser medium higher than that of a solid, would be required.

Unlike X-ray line widths,  $\gamma$ -ray line widths are substantially unaffected by the properties of the medium, with one notable exception: 'recoil-less' emission in a crystal (the Mössbauer effect)5. Indeed, it is just the inability of the medium in which isomeric nuclei are embedded to mix excited nuclear states of high relative multipolarity significantly into ones of lower multipolarity which permits their extraordinary metastability.

Normally,  $\gamma$ -ray line widths are determined by either Doppler broadening (in a high temperature medium) or broadening arising from recoil of the emitting nucleus. The fractional spectral width  $\Delta \nu / \nu$  for thermal Doppler broadening is

$$(\Delta v/v)_{\text{Dopp}} \le 10^{-8} (\theta/A)^{1/2}$$
 (1)

where  $\theta$  is the temperature of the medium (keV), and A is the mass number of the nuclei of interest. The fractional line width caused by recoil of the emitting nucleus is

$$(\Delta v/v)_{\text{recoil}} = \overline{h}\omega/2Mc^2 \approx 10^{-5}k \tag{2}$$

where k is the photon energy in MeV and M is the mass of the recoiling nucleus. Except for the special circumstance of recoil-less emission, the recoil broadening sets a lower bound on the  $\gamma$ -ray line width. In the case of recoil-less emission, the emitting nucleus is bound in a crystal whose Einstein oscillator energy is at least comparable to the kinetic energy acquired by an isolated,  $\gamma$ -ray emitting nucleus. The recoil broadening of such a crystal is negligible because substantial parts of it can take up the recoil momentum. The spectral widths in such cases can be much closer to the natural widths;  $\gamma$ -ray line widths  $\Delta v \sim 10^5$  Hz have been observed for transitions with  $\tau_{\rm spon} \sim 10^{-6}$  s by Mössbauer spectroscopic techniques. By growing very pure crystals with minimal line-broadening lattice stresses respectively. The smaller emission line widths should be attainable with long lived nuclear isomers.

These considerations suggest two possible classes of  $\gamma$ -ray laser: (i) those which operate on allowed (E1 or M1) or weakly forbidden (E2 or M2) transitions, necessarily under vigorous pumping conditions, with  $10^{-5} \lesssim \Delta v/v \lesssim 10^{-3}$ ; (ii) those which operate on forbidden transitions (for example, E3 or M3), in an environment such that  $\Delta v/v < 10^{-15}$  (refs 7 and 8). In (i), the threshold inversion densities will generally be higher than solid densities, so the vigorous pumping must usually be accompanied by compression of the  $\gamma$ -ray laser medium. In (ii), attainment of the small values of  $\Delta v$  needed require recoil-less emission, so the laser medium must be gently pumped, at a temperature less than the Debye temperature<sup>2,3</sup>.

As noted previously<sup>4</sup>, it is not necessary to use cavity-bounding mirrors to produce  $\gamma$ -ray laser action. Indeed, the extremely high minimum fluxes and fluences of a pulsed  $\gamma$ -ray laser would destroy such reflectors. The nature of gently pumped devices may allow the use of Bragg diffraction-type<sup>9,10</sup> cavity mirrors; however, high intrinsic losses ( $\geq 2-3$  dB per pass) make them only marginally useful. We therefore take axial superfluorescence to be a necessary condition for  $\gamma$ -ray laser operation, and (quite conservatively) require

$$\alpha l \gtrsim 30$$
 (3)

where  $\alpha$  is the net gain and l the length of the cylindrical, population-inverted medium. Concurrently, in order to avoid radial superfluorescence, the diameter d of the laser medium must be small compared with its length,

$$d \ll l$$
 (4)

but at least comparable to  $(\lambda l)^{1/2}$  and large relative to  $(\lambda l \alpha)^{1/2}$ , in order to avoid severe diffraction losses. In the absence of losses, equation (3) implies that the net inversion density  $N^*$  must satisfy (L.W., G.C., S. Slutz and G. Zimmerman, UCRL75184, 1974).

$$N^* \gtrsim 30 \left(8\pi/\lambda^2 \left(\Delta \nu \tau_{spon}/l\right)\right)$$
 (5)

where  $\lambda$  is the  $\gamma$ -ray wavelength. Equation (5) is the threshold condition for laser action. We now discuss how this threshold condition might be satisfied for each of the two classes of  $\gamma$ -ray laser.

### Vigorously pumped lasers

We consider, as a possible technique for population inversion, the pumping of nuclear excited states by very high neutron fluxes. The  $\gamma$ -ray laser would consist of a DT thread of milligram mass, loaded with perhaps 1 atom-% of the higher Z nuclei on whose neutron-excited, population-inverted metastable levels the laser will depend for its operation. (Lasing might also take place between levels of population-inverted capture, spallation or fission product nuclei.) Recalling that characteristic 14-MeV neutron excitation/break-up cross sections on such nuclei are 10-24 cm<sup>2</sup>, we note that time-integrated neutron fluxes in compressed-and-ignited fibres of the order of 1024 cm-2 may be obtained over picosecond time scales by compression to densities of 10<sup>4</sup> g cm<sup>-3</sup> (comparable to those proposed for a laser controlled thermonuclear reactor<sup>11</sup>). The corresponding fluxes (> 1035 cm-2 s-1) are more than sufficient to give a high probability of excitation/break-up on time scales characteristic of allowed  $\gamma$ -ray transition of energies < 1 MeV.

The threshold inversion density is  $1.2 \times 10^{28} k^3$  cm<sup>-3</sup>, assuming l=1 cm,  $\tau_{\rm apon}=10^{-12}$  s and taking  $\Delta v/v=(\Delta v/v)_{\rm popp}\simeq 10^{-3}$ , from equation (1), using a typical fusion microexplosion ion temperature of  $\sim 100$  keV and  $A\simeq 10^2$ . We conclude that it is possible to achieve  $\gamma$ -ray laser action with  $k\lesssim 0.2$  in a suitable cylindrical fusion microexplosion for which the space-time power programming of the implosion-driving laser pulse streaked the microexplosion along such a fibre at about the speed of light. Laser pulses of about  $10^5$  J per mm of length would apparently be required to implode and ignite the fusionable material which produces the neutron fluxes needed to pump the emitting nuclei.

Of course, attainment of large inversion densities is not necessarily sufficient for the attainment of laser action, because of  $\gamma$ -ray laser losses in the medium. In order that the gain due to stimulated emission of a vigorously pumped  $\gamma$ -ray laser exceeds the loss due to Compton scattering, the dominant opacity mechanism,

$$\tau_{\rm spon} < 10^{-14}/(\overline{Z}k^3) \, {\rm s}$$
 (6)

where  $\overline{Z}$  is the average atomic number of the laser medium. This is an extremely stringent condition and indeed, there are no  $\gamma$ -ray transitions with k < 0.2 presently known to us which satisfy this condition.

Table 1 Data for a gently pumped γ-ray laser						
k(MeV)	λ (A)	$\lambda^2/8\pi$ (barn)	Δν <sub>max</sub> (Hz)	Δν <sub>mln</sub> (Hz)	ζ <sub>min</sub>	
0.01	1.24	6 × 10°	30.0	1.0	30	
0.05	0.248	$2.4 \times 10^{5}$	1.0	0.01	100	
0.10	0.124	$\begin{array}{c} 6\times10^4\\ 600\end{array}$	0.3	0.001	300	
1.00	0.0124		0.003	0.0001	30	

The Table shows, as a function of  $\gamma$ -ray wavelength, the approximate maximum allowed line width  $\Delta v_{max}$  for a gently pumped  $\gamma$ -ray laser  $(l=3 \text{ cm}, \tau_{spon}=10^4 \text{ s})$ , the internal conversion-limited minimum line width, assuming  $\tau_{spon}=10^4$ ,  $\alpha=10 (0.1/k)^3$ , and the minimum figure of merit  $\zeta \equiv \sigma_{max}/(1+\alpha) \sigma_{abs}$  needed to operate at the maximum allowed width.

### Gently pumped lasers

We have noted that, to exploit recoil-less emission, the excited nuclei must be incorporated in a crystal lattice which is kept sufficiently cool<sup>3</sup>. This suggests that the nuclei must be excited before they are assembled into a crystal lattice. As a practical matter, it seems that nuclei may be excited and then embedded in a recoil-less environment (for example, irradiated in a nuclear reactor, separated from other isotopes and nuclei of the same A and Z but different excitation levels, and then crystallised into a thread of suitable composition) only on time scales > 10<sup>2</sup> s; so we are compelled to use relatively longlived nuclear isomeric states. Moreover, the strengths of chemical bonds of most substances imply that substantially recoil-less environments exist only for  $k \leq 0.1$ . We are therefore most interested in E3 or M3 transitions. We note that the collective nature of these excited nuclear states implies large isomer shifts of electronic spectral lines12, comparable to isotope shifts, which greatly facilitates isomer separation by photochemical/photophysical means (ref. 13 and T. Axelrod, A. Bernhardt, T. O'Leary, J. Marling, R. Keeler and L. W., UCRL74904, 1973).

acoustic isolation would also be required to minimise axial velocity gradients in the medium. Indeed, laser action itself will lead to a relatively slight, variable acceleration of the medium, which is minimal if the system emits collectively. Such super-radiant emission<sup>15,16</sup> is likely ,since the 'cooperative length' for collective de-excitation,  $I_c = (2\pi c \tau_{cp,th} / \lambda^2 N^*)^{1/2} \gg l$ .

Internal conversion is an important limitation on gently pumped  $\gamma$ -ray lasers because of the high multipolarity and relatively low energy of the transitions of interest. For E3 or M3 transitions, the (dominant) K-shell internal conversion coefficient  $\alpha_{\rm K}$  is of the order of 10 for  $k\simeq 0.1$ . Thus our assumed radiative lifetime  $\tau_{\rm apon}$  of  $10^4$  s corresponds to an actual half life  $\tau_{1/2}\simeq 20$  min, which would be the maximum time allowed for isomeric state separation, followed by crystal formation and conditioning. Since  $\tau_{1/2}=(\alpha+1)$   $\tau_{\rm apon}$ , where  $\alpha=\alpha_{\rm K}-\alpha_{\rm L}+\ldots$  is the total internal conversion coefficient, we also have  $\Delta \nu > (\alpha+1)/\tau_{\rm spon}$ .

Photoelectric absorption also severely limits the available  $\gamma$ -ray laser parameter space. The photoelectric cross section per atom in cold matter,  $\sigma_{\rm photo}$ , is of the order of  $Z^4$   $(0.01/k)^3 \times 10^{-26} \, {\rm cm}^2$ . This photoelectric cross section may be quite large

	Table 2 Nuclear isomers considered most promising for gently pumped γ-ray laser action								
Isomer	k(MeV)	λ (Å)	σ <sub>max</sub> (barn)	σ <sub>abs</sub>	α	ζ	τ <sub>1/2</sub> (min)	Class	Production
60mCo	0.059	0.21	$1.8 \times 10^{5}$	120	41	37	10.7	M3	59Co, 100%; 18 barr
<sup>79m</sup> Se	0.096	0.13	$5 \times 10^4$	84	7	112	3.9	E3	<sup>78</sup> Se, 24 % 0.4 barn
81mSe	0.103	0.12	$5.8 \times 10^{4}$	70	9	92	57	E3	80Se, 49%; 0.1 barn
<sup>77m</sup> Br	0.108	0.11	$5.2 \times 10^{4}$	70	6	124	4.2	E3	<sup>77</sup> Se, (p, n) <sup>77</sup> B
99mTc	0.143	0.09	$3 \times 10^4$	70	30	14	350	M4	99 Mo, β- decay

 $\sigma_{max}$  is the approximate maximum stimulated emission cross section possible,  $\sigma_{abs}$  the photoelectric absorption plus Cc mpton scattering cross sections at the transition energy per atom,  $\alpha$  the total internal conversion coefficient and the figure of merit of the isomer,  $\zeta = \sigma_{max}/(1 + \sigma)$   $\sigma_{abs}$   $\tau_{1/2}$  the excited state half life. Also shown are the multipolarity of the radiative decay and the most promising means of production of the isomer, including the fractional natural abundance and thermal neutron capture cross section (in barns) to the isomeric state of its precursor.

\*\*OpenCo\*\*, \*\*PamSe\*\* and \*\*PamSe\*\* are considered especially interesting as they are formed substantially population-inverted.

Maximum values of  $\Delta v$  which allow attainment of threshold  $\gamma$ -ray laser inversion densities below those of a solid (5  $\times$  10<sup>22</sup> cm<sup>-3</sup>) are shown in Table 1, assuming  $\tau_{\rm spon}=10^4$  s and l=3 cm. Although required values of  $\Delta \nu$  are smaller than the smallest so far measured to present<sup>6</sup> (≈ 10<sup>5</sup> Hz), they are much larger than the typical natural widths. It therefore seems reasonable to expect that the required values of  $\Delta v$  may be attained, using carefully prepared crystals (such as metal whiskers which have only a single axial screw dislocation and are effectively perfect) to minimise inhomogeneous line broadening caused by lattice irregularities. Even in such crystals, however, there would be inhomogenities arising from differences between the upper and lower states of the emitting nuclei. For instance, the magnetic dipole-dipole interaction between neighbouring nuclei can be ~ 103 Hz and would result in disasterously large line broadening in even a perfect crystal. Fortunately, such interactions will time-average to much smaller values, producing the required line narrowing, if the nuclear spin temperature is not too low. Such nuclear spin temperatures ( $\gtrsim 10^{-6} \text{ K}$ ) may be attained by many means, if necessary, such as the application of radiofrequency pulses to the  $\gamma$ -ray laser medium<sup>14</sup>. In order to avoid line shifts caused by external magnetic and electric fields, electric field gradients across the laser medium must be kept below 107 V cm<sup>-1</sup> and ambient magnetic field intensities must be  $\lesssim 10^{-4}$  gauss. Also, the second-order Doppler effect and long wavelength phonons may cause significant line broadening in the laser medium, because of strain-inducing thermal gradients and microscopic scale velocity gradients, respectively, unless the ambient temperature is kept below 10 K.

The line shifts induced by gravitational potential difference across the  $\gamma$ -ray laser medium require that these lasers be operated within an angle  $(c^2/gl)$   $(\Delta v/v) \simeq 10^{-4}$  rad of the perpendicular to the local gravitational gradient. Likewise,

compared to the stimulated emission cross section. For example, the stimulated emission cross section corresponding to the maximum allowable line width is  $\approx 10^2$  barn, whereas the photo-electric cross section may be > 103 barn. For a laser operating with bandwidth  $\Delta v$ , the cross section for stimulated emission will exceed that for photoelectric absorption only if  $Z \lesssim 600k^{1/4}$  ( $\Delta v \tau_{spon}$ )<sup>-1/4</sup>. This relation states that, for low photon energies produced by nuclei of high Z, it will be necessary to achieve operating bandwidths approaching the minimum useful widths  $\simeq \alpha/\tau_{ip\cdot n}$ . This suggests a figure of merit for nuclear isomeric candidates:  $\zeta \equiv \sigma_{max}/(1+\alpha)\,\sigma_{abs}$  where  $\sigma_{max}=\lambda^2/8\pi$  and  $\sigma_{at}$  is the sum of the photoelectric and Compton scattering cross sections. This figure of merit is obviously the multiple of the minimum attainable width at which the  $\gamma$ -ray laser will still have a net gain, if the nuclei of the medium are completely populationinverted. Incompletely pure media reduce this figure of merit proportionally to the fractional population inversion. Because  $\zeta$  is, generally speaking, never very large we are not optimistic about proposals<sup>7,8</sup> for using isomers with  $\tau_{1/2} > 10^6$  s.

Photoelectric absorption and internal conversion lead to heating of the gently pumped  $\gamma$ -ray laser medium that might destroy the recoil-less narrowing of the emission line necessary for laser action. For isomeric states with half lives  $>10^{2}\,\mathrm{s}$ , however, the heat generated by internal conversion can be easily removed on the time scales over which laser action occurs by thermal conduction cooling, if the radius of the laser medium is  $\leq 10~\mu\mathrm{m}$ . Heating by photoelectrons in a fibre 1  $\mu\mathrm{m}$  in diameter resulting from a  $\gamma$ -ray laser pulse of length  $\tau_{\mathrm{pulse}}$  s is of the order of  $10^{6}k~(\sigma_{\mathrm{photo}}/\sigma_{\mathrm{stlm}})/\tau_{\mathrm{pulse}}$  eV s<sup>-1</sup> per atom, where  $\sigma_{\mathrm{stlm}}$  is the stimulated emission cross section. A laser medium self-heating rate of  $10^{2}$  eV s<sup>-1</sup> per atom is acceptable (resulting in operating temperatures of 10K for the medium

and implies that  $\sigma_{\rm stlm}/\sigma_{\rm photo}$  must exceed  $10^3 k/\tau_{\rm pulse}$ . Since  $\tau_{\text{pulse}}$  must be shorter than  $\tau_{1/2}$ , the isomeric state half life, this implies that  $\sigma_{\rm stlm}/\sigma_{\rm photo}$  must be greater than  $10^3 k/\tau_{1/2}$ . For  $\tau_{1/2} > 100$  s, this criterion will be satisfied for k < 0.1 if  $\sigma_{stim} > \sigma_{phot}$  , which is the basic net gain condition for  $\gamma\text{-ray}$ lasers (as  $\alpha \gg 1$ ). Thus photoelectric self-heating of the medium during laser action is not a serious problem, if  $\tau_{1/2} \gtrsim 100s$ .

Because of the difficulties of producing small  $\gamma$ -ray line widths in bulk samples, Goldanskii and his colleagues<sup>17-19</sup> see promise only in pulsed (≤ 10<sup>-2</sup> s) nuclear population inversion, where larger values of  $\Delta v$  would be permitted. While we concur with the Goldanskii-Kagan analysis of the requirements on  $\gamma$ -ray laser action in the half life regime 10<sup>3.5±1</sup>, we see these requirements as attainable through extant laser and semi-conductor technologies, and thus do not share their pessimism in these respects. In contrast, scrunity of essentially all nuclear data published through 1972 (rcfs 20, 21 and R. Haight, private communication) does not indicate the existence of even one candidate nuclear species for the nuclear explosion-pumped approach advocated by the Goldanskii-Letokhov school. Such a nucleus must have an excited state half life of > 10<sup>-6</sup> s (for substantial thermal neutron fluxes cannot be generated through fast flux moderation on shorter time scales) but  $\leq 10^{-2}$  s (by the Goldanskii assumptions on the required fractional line width), and have a thermal capture cross section of ≥ 10-20 cm<sup>2</sup> (to prevent overheating of the laser medium associated with thermal neutron fluences of  $\gtrsim 10^{20}$  cm<sup>-2</sup>). Indeed, the <sup>181</sup>Ta nucleus nominated for such a role 17,18 is now known to have several times too small a cross section for neutron capture, though it is the most apt choice we have identified so far. In addition, realistic evaluation of the magnitude of the pulsed time-integrated neutron fluxes to which a medium may be subjected and still show recoil-less narrowing indicates that the estimate of 10<sup>20</sup> cm<sup>-2</sup> is optimistic by at least a factor of three, when heating by scattering of the incompletely moderated (epithermal) neutron spectrum and other nuclear explosion-associated effects (prompt gamma rays, hydrodynamic and thermal conduction energy transport, for example) are considered. The nuclear explosion-pumped  $\gamma$ -ray laser advocated by Goldanskii and collaborators thus fails of attainability by at least an order of magnitude, in the most favourable case for it.

### Realisation of gently pumped lasers

An attempt to operate a  $\gamma$ -ray laser might commence by irradiation of an appropriate target in a high flux nuclear reactor or charged particle accelerator for several nuclear isomeric state lifetimes, so that saturation quantities of isomeric nuclei would be produced. The target might then be rapidly inserted into an atomic beam oven, and the nuclear isomeric state and its daughter ground state nuclei efficiently evaporated into a well collinated atomic beam from which the isomeric state atoms could be routed via any of several photophysical processes (ref. 13, UCRL74904 and ref. 22) into a very small channel (width  $\sim 1 \mu m$ , length 0.1-1cm) machined into a heat sink over which the isomeric beam component rather uniformly loaded. The heat sink would then be rapidly levelled, acoustically isolated, magnetically shielded and cooled to liquid helium temperatures, whereupon y-ray lasing action would commence.

As a particular example, we consider 60Co, whose isomer decays in 10.7 min through a 59-keV M3 transition to a long lived (5.2 yr) ground state and which has the notable property of being born substantially population-inverted, because of the relatively large neutron capture cross section into the isomeric state and the comparatively small statistical weight of this state. This isomer can be produced in milligram quantities on minute time scales by (high flux) reactor thermal neutron irradiation of naturally occurring 59Co. The 60Co and 60 mCo can be separated from <sup>59</sup>Co by γ-cascade-recoil ejection from thin foils at the time of formation. Assembly of the separated isomeric fraction

into a pure, strain-free cylindrical crystal (for example, a metallic cobalt whisker) would be followed by very rapid annealing and purification from impurities generated by β-decay in the panned focal spot of a laser beam, exploiting the very short thermal relaxation times of these whiskers, γ-ray lasing would commence as the required environmental conditions were attained. The relative width that must be achieved to obtain  $\gamma$ -ray laser action for this material is  $\sim 3 \times 10^{-20}$ .

Some nuclei we consider to be candidates for gently pumped  $\gamma$ -ray laser action are given in Table 2. A more complete list of candidate nuclei has been prepared by L. P. Somerville (UCLL Memo 5574-220). Many variations on this basic theme are obvious, some of which are to be preferred for particular nuclear isomers (for example, those with Z different from that of their parent nuclei, such as may be readily produced with accelerator beams).

### Molecular structure

We have briefly reviewed the salient physical conditions limiting  $\gamma$ -ray laser operation, taken note of the two basic types of laser, and described how each type might operate. Such lasers seem to be candidates for producing coherent photon beams with  $h\omega \gtrsim 10$  keV, though quite substantial technological challenges must be met in realising them. Operation of  $\gamma$ -ray lasers with  $h\omega \gtrsim 100$  keV, however, seems exceedingly difficult. Timebandwidth limitations force gently pumped lasers to emit their pulses over multi-second time scales; that is, gently pumped γRLs would be quasi-c.w. sources of extremely coherent and monochromatic radiation with 0.1 Å  $< \lambda < 1$  Å. It seems clear that such sources will be extremely useful in the basic and applied sciences, particularly in the determination of the structure and composition of large molecules.

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# Bacterial mutation affecting T4 phage DNA synthesis and tail production

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A new type of host defective Escherichia coli mutant, hd590, is described. T4+ infection of this mutant results in (1) an abnormally low rate of T4 DNA synthesis that is constant throughout the latent period; (2) relatively low amounts of specific late T4 proteins; (3) the production of empty T4 heads, but not of tails or tail fibres. Two types of T4 mutants, one in T4 gene 31 and the other near T4 gene 39, overcome the hd590 block.

Investigators examining the role of the host cell in phage production have isolated several host mutants that permit phage adsorption but block virus production. Three principal types of 'host defective', hd, cells in which T4 production does not occur have been reported. In the first, T4 DNA synthesis and tail production are normal, but phage capsids do not assemble<sup>1-3</sup>. T4 heads normally assemble on the host cell inner membrane<sup>4</sup>, and one T4 gene product, P31, is known to affect the association of head proteins with the cell membrane<sup>5</sup>. Some T4 mutants in gene 31 can overcome the block in head assembly in the first type of hd bacterium<sup>1-3</sup>.

The second principal type of hd bacterium, a mutant resistant to rifamycin, seems to possess an altered RNA polymerase<sup>6</sup>. T4 infection of this cell results in a slight delay in T4 DNA and late protein synthesis, as well as in a slight delay in the shutoff of host mRNA synthesis. Characteristics of T4 mutants which can overcome this type of hd block have not been reported.

The third type of *hd* bacterium blocks the production of functional tail fibres, producing T4 particles which lack the normal complement of long tail fibres<sup>7</sup>. These fibreless phages can be activated by the *in vitro* addition of long tail fibres.

We describe here a new type of hd mutant, E. coli hd590, which blocks T4 production. After wild-type T4 (T4+) infection of hd590, T4 heads are made, but are not filled with DNA. Tails are not made, and the rate of T4 DNA synthesis is abnormally low for the duration of the latent period. Two types of T4 mutants (T4go590) able to form plaques with high efficiency on hd590 have been isolated and characterised. One type of T4 mutant able to overcome the hd590 block seems to map in gene 31; the other type seems to map in a different region of the T4 genome, near gene 39.

### E. coli hd590 and T4 production

Escherichia coli hd590, a nitrosoguanidine-induced mutant of E. coli B011', was selected for its inability to function as a normal host for T4 and T6 bacteriophages. Uninfected hd590 cells grow well in complex media such as tryptone broth. In a minimal medium, however, such as LSTG (described in Fig. 3 caption) or M9 (glucose, salts, phosphate) hd590 bacteria fail to divide; instead, they form long, fragile filaments. Addition of casamino acids, vitamins, dipeptides, tryptophan or a mixture of deoxynucleosides to the minimal medium did not stimulate

hd590 cell division. Supplementation of minimal medium with both casamino acids (10 µg ml<sup>-1</sup>) and the mixture of deoxynucleosides (5 µg ml<sup>-1</sup>) permitted slow growth and division of hd590 cells.

More than 90% of the hd590 bacteria lost their ability to form colonies within 5 min after addition of T4+ particles at an average multiplicity of infection (MOI) of 5 phages per cell. Although T4+ particles attached to and killed hd590 bacteria with normal efficiency, infection of these bacteria by T4+ particles did not lead to the production of plaque-forming progeny phages (Fig. 1). The block in T4+ production in hd590 was independent of temperature between 20° C and 43° C. In Fig. 1 and in most subsequent figures, T4+ infections

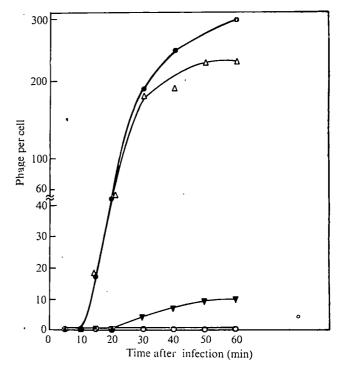


Fig. 1 Phage production and cell killing resulting from T4 infection of E. coli hd590 and E. coli B011'. ♠, B011'; T4+; △, B011'; T4go590-1; ○, hd590; T4+; ▼, hd590; T4go590-1. Mid-log phase bacteria were used in these and in all other experiments described in this paper. T4+ refers to T4D phages. Bacteria growing in broth (10 g Bacto-tryptone, 5 g NaCl, 1,000 ml H<sub>2</sub>O) at 37° C were infected with the appropriate T4 particles (MOI = 5) and 5 min later they were superinfected with the same type of T4 particles (MOI = 5). Viable cell counts were made just before infection and at various times thereafter. Anti T4 serum was added 8 min after infection; 10 min after infection the bacteria were diluted × 10⁴ to prevent inactivation of progeny phages by the antiserum. At various times, samples were removed from the infected cultures; they were exposed to chloroform to lyse the bacteria; they were then plated to determine phage production. Both B011' and hd590 are readily killed by the T4 particles; only B011', however, produces normal yields of T4 particles.

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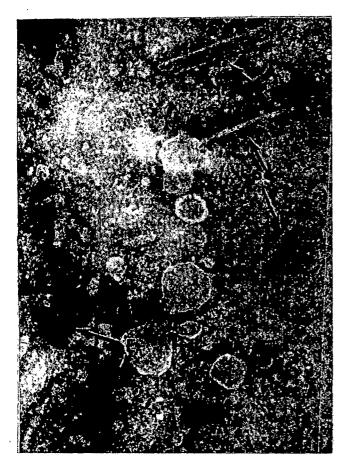


Fig. 2 Electron micrograph of a negatively stained lysate from T4<sup>+</sup>-infected hd590 bacteria. E. coli hd590 cells were infected (MOI = 5) and superinfected (MOI = 5) with T4<sup>+</sup> particles. One hour after infection chloroform and pancreatic DNase (10 g ml<sup>-1</sup>) were added to the culture. Samples of the lysate were then negatively stained with 1% phosphotungstate at pH 7.0 and examined in the electron microscope. The lysate contained empty heads (E) polysheath (PS) and polyheads (PH). (Magnification is approximately × 55,000.)

of B011', the parent *E. coli* strain, are shown as control experiments.

Electron microscopic studies of T4 production after T4+ infection of *E. coli hd* 590 show only empty phage heads, polysheath and polyheads in negatively stained lysates (Fig. 2). Very few tails were observed, and they probably derived from the input phage preparation. The presence of polysheath in the T4+-infected *E. coli hd* 590 lysates indicates that at least some tail proteins were made, although the polysheath occured in a smaller amount than would be expected from a typical T4 mutant blocked in baseplate or tail core assembly.

In agreement with the observations of lysates, electron microscopic examinations of thin sections through T4+-infected  $E.\ coli\ hd590$  cells showed only empty heads in the central regions of the bacteria. In addition, lumps of head precursor material and  $\tau$  particles<sup>4,5</sup> were evident on the bacterial membrane (data not shown). Although empty heads were the predominant head-related structures seen in thin sections, polyheads, resembling tubular  $\tau$  particles, were also visible. Both polyheads and  $\tau$  particles, with their long axes extending towards the interior of the cell, appeared to be bound to the cell membrane.

Using sodium dodecyl sulphate (SDS)-acrylamide gel electrophoresis, we analysed the proteins made after T4+ infection of *E. coli hd590*. Normal T4 infection blocks the synthesis of host cell proteins soon after infection; therefore, T4 proteins were specifically labelled with radioactive precursors. Particular gene products were identified by the use of specific T4 amber mutants which block the synthesis of specific proteins. As Fig. 3 shows, bands representing many late T4 proteins

(such as the major head protein, p23 and the tail sheath protein, p18) were present in the T4+-infected *E. coli hd5*90 sample; however, bands corresponding to the gene 7 and gene 34 products appeared to be very faint or absent. These gene products are normally parts of the phage baseplate and tail fibre structures, respectively<sup>8</sup>. Several other T4 proteins also seemed to be missing or to be made in relatively small amounts. A few bands not present in the control sample (T4+: *E. coli* B) were seen in the T4+: *E. coli hd5*90 infection. It is unclear whether these new bands represent fragments of proteins synthesised in normal T4 infections, host proteins or early proteins which have not been turned off.

Since electron microscopy showed that phage heads were assembled but not filled with DNA in T4+-infected E. coli hd590 bacteria, we compared the rates of T4 DNA synthesis after phage infection of E. coli hd590 and E. coli B011' at 37° C. The determinations were made by measuring the incorporation of H³-thymidine into trichloroacetic acid (TCA)-precipitable material during 2-min pulses given to samples of the infected cultures. As Fig. 4a shows, the rate of DNA synthesis in T4+ infected E. coli hd590 cells was much lower than the comparable rate in infected E. coli B011' cells. The maximal rate of DNA synthesis in T4+-infected E. coli hd590 bacteria was approximately 13% of the maximal rate in similarly infected E. coli B011'.

Figure 4b shows that the amount of newly synthesised T4 DNA within T4+-infected hd590 bacteria was much lower than that in T4+-infected B011' cells. These data also indicate that DNA synthesis in T4+-infected E. coli hd590 began at approximately the normal time, although at a reduced rate which remained constant throughout the latent period (Fig. 4a and b).

In other experiments (data not shown) we found that the DNA which accumulates in T4+-infected hd590 cells sediments more rapidly than the 63S DNA from mature T4 particles. This rapidly sedimenting DNA is characteristic of certain T4 infections in which head filling is blocked<sup>10</sup>.

In an effort to find reagents which could promote T4 production in hd590 cells, we observed that the addition of low concentrations of EDTA to T4+ infected hd590 samples significantly increased the yield of T4 particles (Fig. 5). We added 0.0002 M EDTA to mid-log phase E. coli hd590 cells in tryptone broth at the time of infection with T4+ particles and found a marked increase in the production of progeny phages. This result was essentially the same whether the EDTA was added at the time of infection, 2 min before infection or 8 min after infection. When we increased the EDTA concentration to 0.1 M or higher, we observed no increase in phage production, presumably because the infected cells were damaged by the EDTA.

We also observed that addition of EDTA could alter the level of DNA synthesis in T4+-infected hd590 and B011' cultures (Fig. 5). Although 0.0002 M EDTA had little, if any, detectable effect on DNA synthesis in either sample, higher concentrations of EDTA markedly reduced T4 synthesis in the B011' controls and markedly increased T4 DNA synthesis in the hd590 samples. Thus, the T4+-infected hd590 and B011' samples showed nearly the same levels of DNA synthesis when exposed to 0.0001-0.005 M EDTA.

### T4 mutants

T4 mutants (go590) able to plate on E. coli hd590 occur spontaneously at a frequency of about 10-8. One such phage mutant, designated T4go590-1, was studied in detail. We investigated the physiology of a T4go590-1 infection of E. coli hd590. Although T4+-infected E. coli hd590 cells yielded an average of less than 0.01 progeny phage per cell, T4go590-1-infected E. coli hd590 cells yielded an average of 10 phage particles per bacterium (Fig. 1). This burst size, although sufficient to cause formation of plaques, is lower than would be expected of a completely normal infection. Negative staining of hd590 cells lysed by chloroform 20 min after T4go590-1 infection showed a pattern similar to that seen 20 min after T4+-infection of the cells, with empty heads, polyheads and polysheath. At

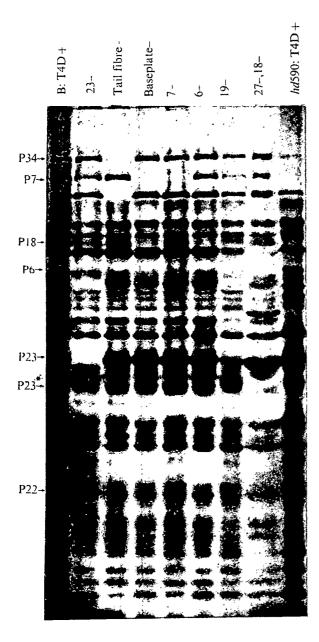


Fig. 3 Autoradiograph of an SDS-acrylamide gel showing the phage proteins in T4<sup>+</sup>-infected hd590 cells. Since E. coli hd590 (su+) do not grow well in minimal medium, hd590 and E. coli B (su) were grown in tryptone broth to 4  $\times$  10<sup>8</sup> cells ml<sup>-1</sup>; the bacteria were pelleted and resuspended in LSTG medium (6.4 ml 0.1 M KH<sub>2</sub>PO<sub>4</sub>, 1.6 ml 0.1 M Na<sub>2</sub>SO<sub>4</sub>, 2.0 ml 0.5 M MgCl<sub>2</sub> 100 ml 10  $\times$  low salts, 100 ml 1.0 M Tris, pH 7.4, 100 ml 20% glucose ,H<sub>2</sub>O to 1,000 ml; 10  $\times$  low salts contains 5.4 g NaCl, 3.0 g KCl, 11.0 g NH<sub>4</sub>Cl, 10 ml 0.1 M CaCl<sub>2</sub>, 10 ml 0.1 M FeCl<sub>3</sub>. H<sub>2</sub>O to 1,000 ml) containing 1% (v/v) tryptone broth. The two cultures were then infected (MOI = 3) and superinfected (MOI = 3) 7 min later with T4<sup>+</sup> particles. A mixture of amino acids— $^{14}$ C (100  $\mu$ Ci ml<sup>-1</sup> for hd590 and 20  $\mu$ Ci ml<sup>-1</sup> for B) and containing 100 ml<sup>-1</sup> for hd590 and 20  $\mu$ Ci ml<sup>-1</sup> for B) and -35S (100 μCi ml-1 for hd590 and 20 μCi ml-1 for B) methioninewas added to the cultures 12 min after infection. The bacteria were pelleted 35 min after infection; the pellets were heated to 100 for 2 min in 1% SDS and 1% β-mercaptoethanol. Other E. coli B cultures, growing in LSTG medium, were infected with various T4 amber mutants and were then treated in the same way as the previously described *E. coli* B sample. The amber mutants used for this gel were T4amH11 (23<sup>-</sup>), T4amXF18 (baseplate<sup>-</sup>), T4amX4E (tail fibre<sup>-</sup>), T4amN102 (6<sup>-</sup>), T4am B16 (7<sup>-</sup>), T4amN120amE18 (27<sup>-</sup>, 18<sup>-</sup>) and T4amE1137 (19<sup>-</sup>). The denatured proteins were run on SDS-acrylamide (10%) slab gels<sup>8</sup> for 5.5 h at 80 V after which the gels were dried. Autoridio for 5.5 h at 80 V, after which the gels were dried. Autoradiographs were then made to allow visualisation of the protein bands. Columns labelled BT 4+ and hd590:T4+ indicate E. coli B and E. coli hd590 respectively infected with T4+ phages. All other columns are infections of E. coli B by the appropriate T4 amber mutant.

later times, however, intact phage particles appeared in the T4go590-1-infected samples, but not in the T4+-infected E. coli hd590 samples.

To determine whether the pattern of phage DNA synthesis in T4go590-1-infected E. coli hd590 differs from that observed after T4+ infection, we measured thymidine incorporation into TCA-precipitable material at various times after the infection of cultures of E. coli hd590 bacteria with either T4go590-1 or T4+ phages. The data from this experiment (Fig. 4a) suggest that until 26 min after infection, the rates of DNA synthesis in T4go590-1 and in T4+-infected E. coli hd590 bacteria are similar. After 26 min, however, the rate of phage DNA synthesis in the T4go590-1 infected culture started to increase whereas there was no comparable increase in the T4+-infected E. coli hd590 culture. Sixty minutes after infection, the rate of DNA synthesis in the T4go590-1-infected hd590 culture is about double the rate in the T4+-infected hd590 culture.

The map position of T4go590-1 was determined from analysis of a series of crosses performed as described before<sup>11</sup>. Genotypes of the progeny phages from such crosses were identified using method II of Doermann and Boehner<sup>12</sup>, but adopting modifications appropriate to the particular mutants involved (details in legend for Table 1). The first cross used T4go590-1 as one parent and, as the second, a strain carrying mutations in the following 12 genes: e, 5, 11, 24, 48, 34, 37, ac, rIIB, 39, 43, and 46. Data from more than 1,000 progeny phages showed that the recombination frequency of go590-1 with other markers was lowest with amN85 (gene 48) and increased progressively for markers in both the clockwise and the counterclockwise directions from gene 48. The maximum value was attained with amN130 (gene 46). In cross 2 therefore, the second parent was replaced by a phage with markers in genes 25, 26, 51, 27, 29, 48, 31 and 34. Although 1,050 progeny were scored and both complementary recombinants found with all other markers, not a single recombinant was found for go590-1 and am N54N (gene 31). Cross 3 involved parents with makers in 7 genes (29, 48, 30, 31, 32 and 34), and analysis of 1,118 progeny phages again failed to uncover a single recombinant between go590-1 and amN54 although all other recombinant classes were recovered with reasonable frequencies. Thus, among more than 2,000 cross-progeny phages neither the doubly mutant nor the wild-type recombinant for these two markers was found. It must be concluded that go590-1 is very close to amN54 in gene 31 or perhaps represents a multisite mutation whose ends overlap the site of the amN54 mutation.

With the possibility of a multisite mutation in mind, recombination between go590-1 and a second amber mutation in gene 31 (amNG71, supplied by W. B. Wood) was examined. Both wild-type and doubly mutant recombinants were found. The cross was repeated with the mutant markers in coupling. The marker amH39 (gene 30) was also included in both crosses in the hope of ordering the mutations by three-point tests. Parallel crosses were made with go590-1 replaced by amN54. The results of the four crosses are summarised in Table 1. If go590-1 is due to a multisite mutation it might be anticipated that recombination between it and amNG71 is less than between am-N54 and amNG71 because the effect of a multisite mutation might reduce recombination beyond amN54 in either direction. The results show no evidence for that hypothesis. We also note that the data suggest that the order of markers is that used in Table 1, namely amH39-amN54 (or go590-1)-amNG71. High negative interference reduced the effectiveness of the threepoint test here as in other cases of close linkage<sup>11</sup> so that the ordering of the markers in these crosses is less than completely

Four T4 mutants of independent origin able to grow on hd590 were isolated by Barbara North and Gary Thorgaard, who permitted us to comment here on their results. Each of these strains simultaneously acquired the ability to multiply also on the bacterial mutant hdF isolated by Revel<sup>13</sup>. All of these strains differ from go590-1 which is unable to make plaques on hdF. H. R. Revel (personal communication) has shown that her T4goF

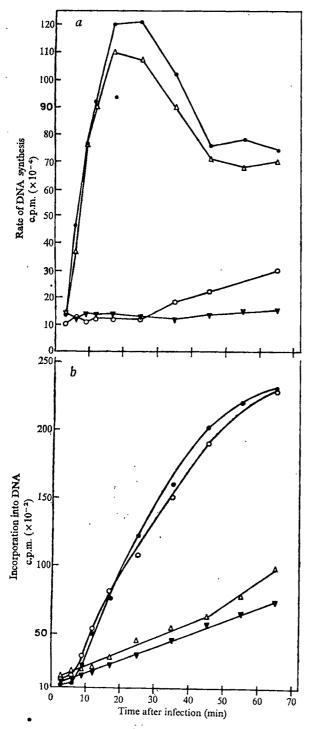


Fig. 4 Synthesis of DNA after T4 infection of hd590 and B011'. Bacteria were grown to 5 × 10<sup>8</sup> ml<sup>-1</sup> at 37° C in tryptone broth which contained 100 μg ml<sup>-1</sup> deoxyadenosine and 0.5 μg ml<sup>-1</sup> thymidine. The cells were then infected with the appropriate T4 particles (MOI = 5) and 7 min later they were superinfected (MOI = 5) with the same type of phage. a, To measure rates of DNA synthesis, at various times after infection, 1 ml samples were removed from the culture; they were added to 10 μCi <sup>3</sup>H-thymidine. Two minutes later ice-cold TCA plus thymidine was added to a concentration of 10% TCA, 50 μg ml<sup>-1</sup> thymidine. After 10 min the material insoluble in the cold, 10% TCA was collected on glass fibre filters which were washed twice with 5% TCA containing 25 μg ml<sup>-1</sup> thymidine and then twice with ethanol. The filters were dried and counted in a liquid scintillation counter. Δ, B011': T4+; ♠, B011': T4go590-1; ▼, hd590: T4+; ♠, hd590: T4+go590-1. b, To measure total TCA-precipitable DNA <sup>3</sup>H-thymidine (10 μCi ml<sup>-1</sup>) was added to the bacterial cultures at the time of infection. At various times thereafter 10 μl samples were placed on filters which were then immersed in ice-cold 10% TCA. The filters were then treated as described in (a). ♠, B011': T4+; ♠, B011': T4go590-1; ▼, hd590: T4+; ♠, hd590: T4+; ♠, hd590: T4+go590-1.

- 7

mutants map in the vicinity of gene 39. One of the new go590 mutants has been shown to map far from gene 31 and near gene 39, having yielded 4.4% recombinants with amS2 (gene 39). It seems clear that either a mutation in gene 31, a gene essential for capsid development, or a mutation in another region separated from gene 31 by one-fourth of the T4 chromosome, can compensate for the alteration of hd590 which prevents wildtype T4 from multiplying in it.

#### **Implications**

The data presented here describe a host defective bacterium, *E. coli hd5*90, in which the production of T4+ phages is blocked. Unlike other T4 *hd* bacteria that have been reported<sup>1-3,6,7</sup>, T4+infected *hd5*90 cells show: (1) an abnormally low rate of phage DNA synthesis, a rate which is constant throughout the latent

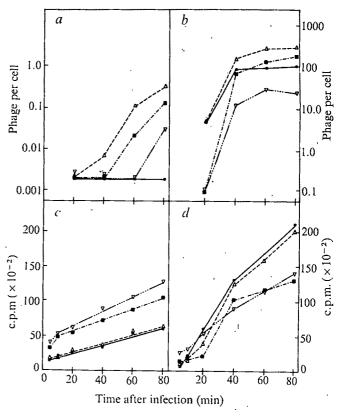


Fig. 5 Effect of EDTA on T4<sup>+</sup> production in hd590 and in B011'. Bacteria were grown to  $5 \times 10^8$  cells ml<sup>-1</sup> at 37° C in tryptone broth which contained 100 µg ml<sup>-1</sup> deoxyadenosine and 0.5 µg ml<sup>-1</sup> thymidine. EDTA was then added to the appropriate final concentration and the cells were infected with T4<sup>+</sup> particles (MOI = 3); 5 min later the cells were superinfected to inhibit lysis with additional phages (MOI=3). In experiments to measure phage production, anti T4 serum was added to the cultures 10 min after infection to inactivate unadsorbed T4 particles, and 5 min later the cultures were diluted  $10^3$ -fold to prevent inactivation of progeny phages. At various times thereafter, samples were withdrawn from the cultures, lysed with chloroform, and assayed for plaque-forming T4 particles. Experiments to measure DNA synthesis were performed as described in Fig. 4b. a, Phage production in hd590; b, phage production in B011'; c, DNA synthesis in hd590; d, DNA synthesis in B011'. , No EDTA;  $\triangle$ , 0.2 mM EDTA;  $\square$ , 1.0 mM EDTA;  $\triangledown$ , 5.0 mM EDTA.

period; (2) relatively small amounts or the absence of specific late T4 proteins; (3) empty heads, but no tails or tail fibres, in lysates. The absence of tails and tail fibres is probably due to the absence of the products of genes 7 and 34 in T4+-infected hd590 samples, gene products that are parts of the T4 baseplate and tail fibre, respectively. Two types of T4 mutants, one which appears to map near T4 gene 39 and one which appears to map

Table 1 Location of go590-1 in the T4 genetic map							
Progeny genotypes $am_1am_2am_3 + + +$	Cross 1 5 12	Cross 2 58 95	Progeny genotypes $am_1 + am_3 + go +$	Cross 3 7 20	Cross 4 51 79		
$am_1am_2+ + am_3$	508 567	1* 5*	$am_1 + + go am_3$	438 595	3* 4*		
$am_1 + + am_2am_3$	5* 1*	446 577	$am_1 go + + + am_3$	9* 2*	551 487		
$am_1 + am_3 + am_2 +$	52 39	4 4	$am_1 go am_3 + + +$	<b>5</b> 7 61	6 6		
Total phages identified	1,189	1,190		1,189	1,187		

Crosses were made in *E. coli* CR63 following the methods outlined by Chase and Doermann<sup>11</sup>. The genotypes of individual progeny phages were identified as described by Doermann and Boehner<sup>12</sup>, but with the following technical modifications to accommodate peculiarities of specific mutants. The wild-type and mutant alleles of go590-1 were distinguished by replicating on to a test plate in which the 5ml soft-agar overlay contained  $2.5 \times 10^7$  wild-type T4 per ml. 0.4 ml of a 20:1 mixture (v/v) of aerated overnight broth cultures of hd590 and CR63/s, and streptomycin (hd590 is resistant to streptomycin, having been derived from the streptomycin-resistant strain 011'). To differentiate amH39 from its wild-type allele a streptomycinresistant mutant was isolated from the *E. coli* strain *lop8lig2* isolated by Gellert and Bullock<sup>14</sup> and supplied by Hillard Berger. The soft agar overlay of the replica plates contained, in addition to streptomycin,  $10^7$  amH39 phage per ml as well as 0.4 ml of a 59:1 mixture (v/v) of overnight cultures of lop8lig2/s and CR63/s. The amNG71 tests were made using streptomycin, a 20:1 mixture of B/s and Cr63/s, and  $2.5 \times 10^7$  amNG71 per ml soft agar. In the Table,  $am_1$ ,  $am_2$ , and  $am_3$ represent amH39, amN54, and amNG71 respectively. Asterisks mark the classes in each cross presumed to be double crossovers on which the order of the markers has been based. The parental classes are, of course, the most frequent pair.

in T4 gene 31, can overcome the hd590 block. T4 gene 39 seems to function to promote normal T4 DNA synthesis<sup>15</sup>; T4 gene 31 has been thought to function specifically in T4 head assembly. The 31 mutant, T4go590-1, is different from those other mutants in gene 31 which are able to produce phage in the hd cells which block T4 head morphogenesis<sup>1-3</sup>. This is the first report, to our knowledge, of a T4 gene 31 mutant which affects the synthesis of T4 DNA and late proteins.

Although we could not transfer the hd590 character into another cell, two lines of evidence suggest that the block in T4 production results from a single host cell mutation. First, mutant T4 phages, such as T4go590-1, which can overcome the hd590 block appear to be mutated in a single gene. Therefore, one specific phage alteration can affect T4 DNA synthesis, protein synthesis and head filling in hd590. Second, preliminary studies suggest that among bacterial revertants the various aspects of the hd590 character are lost jointly, as would be expected if the hd590 phenotype were due to a single mutation.

If the hd590 character results from a single alteration of the host cell, what is its molecular basis? The low rate of T4 DNA synthesis in hd590 cells does not seem to result from a failure to synthesise T4 DNA polymerase (gene 43 product<sup>16</sup>). In extracts of T4+-infected hd590 cells, we find normal levels of T4 polymerase activity in vitro (unpublished data of D.S., B. Fansler and L. D. S.). Furthermore, sedimentation profile analysis (unpublished data of McLaughlin and L. D. S.) indicates that the apparent low rate of DNA synthesis in T4+-infected hd590 cultures is not due to degradation of the T4 DNA.

In normal T4+ infection, late mRNA production is coupled with DNA synthesis<sup>17</sup>. Since T4 DNA synthesis is abnormal in hd590, the synthesis of late mRNA may also be abnormal, and, if so, may be responsible for the reduced synthesis of specific late T4 proteins in T4+-infected hd590 cells. Alternatively, the hd590 defect might specifically prevent assembly of tail fibres and baseplates, thereby leaving the products of genes 7 and 34 unassembled, and sensitive to proteolytic activities within the infected cell. It seems unlikely that the absence of various proteins from T4+-infected hd590 cells is simply due to an increased host cell protease activity, since T5 and T7 phages plate with normal efficiency on hd590.

Available evidence suggests that the altered host cell component in hd590 may be associated with its inner membrane. This suggestion is based on two observations. (1) Low concentrations of EDTA, which primarily seem to affect the bacterial membrane<sup>18</sup>, stimulate T4+ DNA synthesis and phage production in hd590. (2) A specific T4 mutant in gene 31, T4go590-1, can overcome the hd590 block in T4 production. T4 gene 31 product normally functions in the assembly of the T4 capsid, at the level of cell membrane interaction with T4 head proteins<sup>4,5</sup>. Thus, either EDTA or a specific mutant T4 gene 31 product, both of which may interact with the host cell inner membrane, can stimulate T4 DNA synthesis and phage production in E. coli hd590.

Georgopolous and Herskowitz<sup>18</sup> have described hd mutants called GroP- which affect  $\lambda$  DNA synthesis. Specific  $\lambda$  mutants in gene P can overcome the GroP-block, presumably by making an altered P product that can interact with the altered host cell component. The GroP- defect has been traced to an alteration of the DNA 'B' gene of E. coli<sup>19</sup>. The low level of T4 DNA synthesis in hd590 cells might also be due to an alteration of the bacterial DNA synthesis machinery. This suggestion would imply a greater dependence of T4 on the E. coli DNA replication system than has heretofore been assumed, since T4, in contrast to \(\lambda\), codes for more than 20 phage-induced enzymes of DNA metabolism<sup>20</sup>—including DNA polymerase<sup>16</sup>, polynucleotide ligase21, polynucleotide kinase22 and various deoxyribonucleases23.

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# Evolution of C-type viral genes: inheritance of exogenously acquired viral genes

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Genes related to the nucleic acid of an endogenous domestic cat C-type virus (RD114) are found in the cellular DNA of anthropoid primates while many members of the cat family Felidae lack these sequences. Endogenous viruses from primates are thus concluded to have infected and become part of the germ line of an evolutionarily distant group, the ancestors of the domestic cat.

We have evidence that C-type viruses have in the past been transferred between vertebrate species that are only remotely related phylogenetically. Our data suggest that viral genes from one group of animals can give rise to infectious particles that not only can integrate into the DNA of another species, but can also be incorporated into the germ line and be transmitted as cellular genes. We have found that the RD114/CCC group<sup>1-3</sup> of endogenous feline viruses originated from a primate C-type virus horizontally transmitted to some species of *Felis* relatively recently in feline evolution.

The chromosomal DNA of various mammalian species contains multiple copies of nucleic acid sequences that can code for the production of endogenous C-type viral particles<sup>4</sup>. With <sup>3</sup>H-DNA transcripts prepared from the endogenous baboon C-type viruses<sup>5,6</sup>, partially homologous sequences have been detected in the cellular DNA of Old World monkeys, apes and man<sup>7,8</sup>. These virogenes have evolved for at least 30 Myr among the anthropoid primates in a fashion that correlates closely with the taxonomic relatedness of the species8. Domestic cat DNA contains C-type virogenes9-12 which can give rise to RD114/CCC viruses<sup>2,3</sup>. By several criteria, including nucleic acid sequence homology<sup>5,7</sup> and antigenicity of the polymerase and the p30 protein<sup>6,13,14</sup>, these viruses are related to, but distinct from, the baboon viruses. The unexpected relatedness of the endogenous cat and the endogenous baboon viruses suggested that they had a common origin.

#### RD114-related sequences in cats and primates

The origin of the RD114/CCC cat viruses can be clarified by examining the cellular DNAs from other members of the Felidae, which consist of 36 species belonging to six genera. The use of labelled unique sequence (non-repetitive) cellular DNA to compare the extent of nucleotide sequence divergence among species has shown that the cellular DNAs of the species examined have a nucleic acid sequence homology consistent with classical phylogeny<sup>15</sup>. Hybridisation of labelled uniquesequence domestic cat DNA to the DNA of other Felidae (Fig. 1b) showed that they are all closely related. The DNA of another carnivore (dog) contains much less sequence homology to domestic cat cellular DNA. All modern carnivores are believed to have had a common ancestor approximately 35 Myr ago; the close relationship observed between the DNA of all members of the Felidae indicates that they represent a more recent radiation (<15 Myr ago). The cellular DNA of the cats examined thus contains nucleic acid sequences closely related to each other, as are those of the Old World monkey subfamily Cercopithecinaes. The Felidae and the Cercopithecinae, however, lack detectable reciprocal DNA sequence homology.

Hybridisation of cat cellular DNAs to a <sup>3</sup>H-DNA transcript prepared from RD114 virus (Fig. 1a) showed that homology was greatest with the domestic cat (Felis catus) DNA; at the maximal extent of the reaction, approximately 80% of <sup>3</sup>H-DNA was resistant to digestion with the single-strand specific nuclease, S<sub>1</sub>. The cell DNA from three other species of cats, European wildcat (F. sylvestris), sand cat (F. margarita) and jungle cat (F. chaus), also contained nucleic acid sequences homologous to the RD114 <sup>3</sup>H-DNA probe. Under our hybridisation conditions, however, no other species of Felis or any other feline genus had base sequences related to RD114. In contrast, the DNA of all primate species whose unique sequence cellular

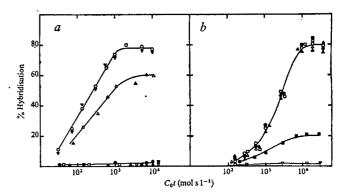


Fig. 1 Hybridisation of RD114 C-type viral ³H-DNA probe and domestic cat unique sequence cellular ³H-DNA to feline cellular DNAs. The ³H-thymidine-labelled DNA probes were synthesised from detergent-disrupted C-type virus in the presence of actinomycin D as described¹³. The specific activity of the ³H-DNA was 1.8 × 10° c.p.m. µg⁻¹. The ³H-DNA probes contained 63–72% of their respective 70S viral RNA sequences at a ³H-DNA: ³²P-viral RNA molar ratio of 1.5 (ref. 7), indicating that they contain most of the sequences present in viral RNA and that these sequences are in proportions similar to their content in 70S RNA. Cellular DNA was extracted from tissues and cell lines as described². All DNAs were sonically treated so as to yield a mean size of 6-85 (the size of the ³H-DNA probes) as determined by centrifugation on alkaline sucrose gradients². DNA: DNA hybridisations were incubated at 65° C in reaction mixtures containing 0.01 M Tris, pH 7.4, 0.75 M NaCl, 2 × 10⁻³ M EDTA, 0.05% SDS, 10,000 to 20,000 c.p.m. of ³H-DNA and 1-3 mg of cellular DNA per ml. Hybridisations were started by heating the mixtures to 98° C for 10 min, cooling on ice to 4° C and incubating at 65° C. At various times (from 15 min to 96 h), 0.05 ml portions were removed and frozen at −80° C until digested with the single-strand-specific nuclease, S₁, as described¹³. C₀t values (C₀ is the concentration of cellular DNA in mol 1⁻¹, and t is the time in seconds) were calculated as suggested by Britten and Kohne²o as (A₂₀o ml⁻¹) / 2 × h, and corrected to a monovalent cation concentration of 0.18 M²¹. a, Annealing of ³H-DNA probe prepared from RD114 grown in RD cells¹ to DNA extracted from: (△) ionspleen; (♠) leopard cat spleen; (□) tiger liver; (△) lion spleen; (♠) dog liver; (√) human spleen. b, Annealing of unique sequence domestic cat cellular ³H-DNA to the cellular DNA of various cats. The DNA of a domestic cat cell line was labelled with ³H-thymidine and extracted². Unique sequence cellular DNA was isolated by removing the highly reiterated se

DNA is homologous (or nearly so) to baboon cellular DNA hybridised extensively to the baboon C-type virus probe<sup>7,8</sup>.

The thermal stability of nucleic acid hybrids is an index of base-pair mismatching between the strands of a double-strand DNA molecule. Mismatching yields hybrids that melt at a lower temperature; the overall effect of mismatched base-pairs on the thermal stability is approximately 1° C for 1% altered base-pairs<sup>16</sup>. Thermal stability data obtained with 13 species representing six feline genera by using either labelled nonrepeated domestic cat cellular DNA or 3H-RD114 viral DNA as a probe are shown in Table 1. Although the overall uniquesequence cellular DNAs of all the cat species were highly homologous (the largest difference in thermal stability is less than 2° C), only four species of Felis contained RD114-related sequences. There were no sequences related to RD114 3H-DNA in two carnivore species (mink and dog) or in mouse or cow cell DNA. Nucleic acid sequences related to RD114 were found in six species of Old World monkeys. Among the apes,

sequences related to RD114 or CCC <sup>3</sup>H-DNA probes can be detected readily in chimpanzee and gorilla cellular DNA. For reasons not yet clear, greater homology has been observed consistently with these two apes as compared with gibbon and human DNA. Sequences related to the baboon C-type virus, while found in all apes, are also detected more readily in chimpanzee and gorilla DNA<sup>8</sup>. Hyman DNA has been examined for RD114-related nucleic acid sequences with negative results<sup>9-12</sup>. The lack of detectable sequence homology of human DNA with RD114 is not due to an absence of RD114-related information. In support of this conclusion, p30 protein partially related to RD114 p30 can be found in tissues of humans<sup>17</sup>, as well as baboons<sup>13</sup> and other Old World monkevs<sup>18</sup>.

Melting curves obtained with a single-stranded <sup>3</sup>H-DNA transcript of RD114 RNA hybridised to the DNA of domestic and jungle cat and to the DNA of Old World monkeys are shown in Fig. 2a. The RD114-related sequences in these

Table 1 Nucleic acid homology and thermal stability between domestic cat unique sequence cellular DNA and RD114 viral DNA and the DNA from various species

		DIVA Holli various species	I Iniana a	aguar co		F
			Unique sequence domestic cat Cellular <sup>3</sup> H-DNA‡		RD114/C 3H-D	NA§
Species*		Geographical range	% hybrid	$\Delta T_{ m m}$	% hybrid	$\Delta T_{ m m}$
	Carnivores					
	Felidae <i>Felis</i>					
	domestic cat (F. catus)	North Africa†	100	< 0.5	100	< 0.5
	European wildcat (F. sylvestris)	Europe, Asia Minor	> 95	< 0.5	95	< 0.5
	sand cat (F. margarita)	North Africa, Arabia	> 95	< 0.5	81	< 1.0
	jungle cat (F. chaus)	Nile Valley, Asia Minor	> 90	< 1	70	1.5
	leopard cat (F. bengalensis)	Southeast Asia	> 90	< 1	< 2	
	golden cat (F. temmincki)	Southeast Asia	> 90	< 1	< 2	*******
	geoffroy's cat (F. geoffroyi) Lynx	South America			< 2	
	caracal cat (L. caracal)	Africa, Asia Minor	> 90	< 1	. <2	
	bobcat (L. rufus)	North America	> 90	< 1	< 2	
	Panthera					
	lion (P. leo)	Africa, Asia	> 90	< 1	< 2	minerals.
	tiger (P. tigris)	Asia			< 2	*******
	Leo	A.C.i A.i.	- 00	. 1	-2	
	leopard ( <i>L. pardus</i> ) <i>Uncia</i>	Africa, Asia	> 90	< 1	< 2	
	snow leopard (U. uncia)	Central Asia	> 90	< 2	< 2	***************************************
	Acinonyx				- <del>-</del>	
	cheetah (A. jubatus)	Africa, Asia Minor			< 2	-
	Other carnivores	·				
	mink		23	10	< 2	- Parker
	dog		19	11	< 2	
	Primates					
	New World monkeys (Cebidae)		_		_	
	capuchin		2	-	< 2	and the contraction of the contr
	Old World monkeys (Cercopithecoidea)		•		20	10.5
	baboon		2	-	20	10.5
	mangabey		3 2 3		15	11.0
	patas		2		11	11.0
	African green		3 1		. 11 . 18	11.0
	macaque: rhesus stumptail		1		· 17	11.0 ° 10.5
	Apes and man (Hominoidea)					
	chimpanzee `		2	-	7.5	NT
	gorilla		2		8.5	NT
	gibbon		1		< 2	Program
	human		1		< 2	
	Other mammals		_		_	
	mouse		2	-	< 2	
	cow		2		< 2	

<sup>\*</sup> Cellular DNA was extracted from various tissues of the species listed.

† Since its domestication this cat has travelled with man all over the world. All breeds of the domestic cat (for example, Burmese, Siamese and Abyssinian) belong to the same species.

§ A  $^{3}$ H-DNA probe prepared from RD114 virus grown in a human cell line was used for the hybridisations to feline DNA, and a  $^{3}$ H-DNA probe prepared from CCC virus grown in a canine thymus cell line (FCf2Th) for the hybridisations to primate DNA. The  $T_{\rm m}$  of the homologous RD114: domestic cat DNA hybrid was 88.5° C.

NT, not tested.

<sup>† &</sup>lt;sup>3</sup>H-thymidine-labelled unique sequence domestic cat cellular DNA was hybridised to the cellular DNA of the various species as described in Fig. 1. The % hybrid is the saturating normalised value obtained after digestion of the hybrids with  $S_1$  nuclease. The actual final extent of domestic cat cell DNA hybridisation to the cellular <sup>3</sup>H-DNA probe was 73%. The temperature at which 50% of the hybrids are dissociated  $(T_m)$  was 85° C for the homologous domestic cat: domestic cat hybrid; the  $\Delta T_m$  is the difference in  $T_m$  between the other DNA hybrids and the  $T_m$  of the homologous hybrid. The  $T_m$  of hybrids that hybridise less than 3% cannot be determined because of insufficient homology. § A <sup>3</sup>H-DNA probe prepared from RD114 virus grown in a human cell line was used for the hybridisations to feline DNA, and a <sup>3</sup>H-DNA

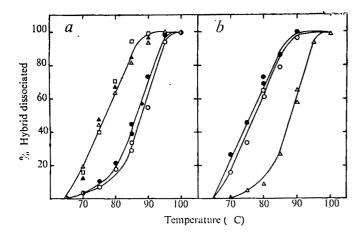


Fig. 2 Thermal stability of hybrids formed between baboon viral 3H-DNA and RD114 3H-DNA and various feline and primate cellular DNAs. a, An RD114 3H-DNA probe was hybridised to DNA extracted from: (O) domestic cat liver; (●) jungle cat spleen; (△) baboon lung; (▲) patas liver; (□) rhesus liver. b, A baboon C-type <sup>3</sup>H-DNA probe was hybridised to DNA extracted from: (△) baboon lung; (○) domestic cat liver; (●) jungle cat spleen. After hybridising to a Cot of 104, aliquots (containing 400 to 700 c.p.m.) were heated for 5 min in 0.75 M NaCl at the indicated temperatures. The amount of hybrid remaining was determined with the singlestrand-specific nuclease, S1.

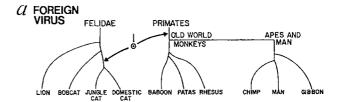
primates melt at approximately 11° C lower than the homologous hybrid. The lower thermal melting points obtained with the Old World monkey DNAs indicate that the partial homology detected is not the result of a strict preservation of discrete continuous segments of the viral genomes in these species, but rather of a more general base-pair substitution throughout the genome. Figure 2b shows the reciprocal experiment: the melting curves obtained when a single-stranded 3H-DNA transcript of baboon C-type viral RNA was hybridised to the DNA of baboon and domestic and jungle cat. Again, baboon type C related sequences in the cellular DNA of the two cats melt at about 11° C lower than the homologous hybrid.

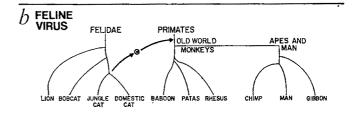
#### Models for viral transmission among species

When the virogenes of two species are more closely related to each other than are the cellular genes, one must suspect horizontal transmission and subsequent perpetuation of the viral genes through the germ line. Figure 3 shows models which could account for the data. A virus of unknown origin infects the ancestors of certain cats and primates in model a. In model b, a virus ancestral to the four species of cats described above infects an ancestor of the Old World monkeys and apes. In model c, the converse occurs: an endogenous primate virus infects a recent ancestor of the domestic cat. If RD114 were an endogenous Felidae virus, there should be sequences related to it in the cellular DNA of all Felidae. Since RD114-related nucleic acid sequences can be found in all Old World monkey and ape tissues but in just four closely-related species of Felidae, we conclude that RD114 is a primate C-type virus, as shown in model c. The greater homology to RD114 nucleic acid sequences in Old World monkey cell DNA than in ape DNAs (Table 1) shows that the transmission occurred after the Old World monkeys and the apes diverged (arrow II or III). Since all the Old World monkey DNAs show comparable levels of homology to RD114, model cII is most likely.

The cats of the genus Felis that contain DNA sequences related to RD114 are from the Mediterranean basin (Table 1) while those from south-east Asia and the New World lack these sequences. The larger African cats (leopard and lion) also do not contain RD114 sequences in their cellular DNA. Therefore, the successful transmission of gene sequences was subsequent to the major radiation of Felidae and limited to one geographic

C-type viruses have thus under natural conditions transferred genetic information between species that are only remotely related phylogenetically. We have shown here that viral genes from one group of animals can give rise to infectious particles that not only can integrate into the DNA of animals of another species, but can also be incorporated into the germ line. There may well be other examples among C-type viruses of germ line inheritance of exogenously acquired viral genes.





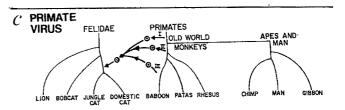


Fig. 3 Schematic representation of models which could account for the presence of related virogene information in primates and domestic cats. a, Common foreign virus infection of ancestors of both Old World monkeys and domestic cats. b, Infection of the ancestors of Old World monkeys and apes by a cat virus. c, Infection of the ancestors of domestic cats and jungle cats by a primate virus. Three possible times for the infection are shown and discussed in the text: (I) before the Old World monkeys and apes diverged (> 30 Myr ago); (II) before the Old World monkeys diverged (> 5-10 Myr ago); (III) recently by a baboon C-type virus (<10 Myr ago).

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# letters to nature

# Equivalence principle: 60 years of a misuse?

Ever since Einstein introduced the equivalence principle and used it, in conjunction with the Doppler effect, to derive the gravitational redshift of spectral lines1, this argument has been an ingredient of many<sup>2-6</sup> (though not all<sup>7-11</sup>) expositions of relativity theory. We show here that on this point Einstein was in error, although the result found is correct (to first

Briefly, the usual reasoning runs that a source S emits a photon of frequency  $v_s$  towards a detector D when both are relatively at rest at distance I apart. At the same instant the detector commences a relative motion with constant acceleration g relative to, and away from, S. D receives the photon at time  $t \sim l/c$  when it has acquired a relative velocity  $v = gt \sim gl/c$ . The observed frequency  $\nu_{\scriptscriptstyle D}$  at the detector is therefore subject to a Doppler redshift and is given to first order by

$$v_{\rm D} = (1 - v/c)v_{\rm S} = (1 - gl/c^2)v_{\rm S}$$
 (1)

The frequency of a photon is therefore redshifted as it ascends a gravitational field if, by the equivalence principle, the acceleration is replaced by a gravitational field g acting in the opposite direction, that is from D to S. Previously synchronised clocks therefore run slower the deeper they are placed in a gravitational field and hence space-time acquires a non-Minkowskian metric.

This reasoning uses the first order longitudinal Doppler effect which is a part also of Newtonian physics. Since 'fictitious' forces, and the equivalence principle, can be considered to be also part of Newtonian physics the reasoning just offered leads to a gravitational redshift and a timetransformation in Newtonian physics. This is an absurd conclusion, since Newtonian physics operates with an absolute time.

The cause of the trouble lies in the application of the equivalence principle. All physical measurements must be imagined to be made with respect to the accelerated frame, but the Doppler formulae involve two different frames (normally both inertial, although generalisations are possible). We have gone back to fundamentals and considered pairs of signals (which is how the Doppler formulae are deduced) and obtained an argument (too lengthy to be set out here) which satisfies the constraint that the gravitational redshift is zero in Newtonian physics, but satisfies equation (1) to first order if special relativity is used and g is interpreted to be a gravitational acceleration. Our argument for Newtonian physics uses a particle picture of light and attributes different velocities to the two signals.

A manuscript giving details will be submitted shortly. Here we merely observe that as a result of what has been said, it is not to be expected that the incorporation in the usual Doppler shift argument of a more accurate Doppler shift expression can be of relevance to the gravitational redshift, as recently proposed12. This argument leads to a relationship between measurements in the inertial frame and a non-inertial frame, and is thus not suitable for a discussion of the gravitational redshift. What is achieved in ref. 12 is the following: If in the argument at the beginning of this letter D is undergoing a constant proper acceleration g away from S, then equation (1) holds exactly. This is a correct result for Doppler shifts arising in hyperbolic motion.

We note, furthermore, that in a gravitational redshift formula which is exact within the framework of special relativity and the equivalence principle (that is, for flat space-times), the way in which lengths are imagined to be measured affects the result. If *l* is the proper or ruler distance between the stationary source and detector in a gravitational field g(x), which can be a function of distance and which is parallel to SD, then we find that equation (1) is indeed exact in the above sense, g being the gravitational acceleration at D. If, however, either coordinate distance or radar distance are used we find that equation (1) holds only to first order.

These results are clearly relevant to ref. 12 and any discussion it may precipitate. N.T.B. thanks the Science Research Council for a research studentship.

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### Gravitational searchlight and its astrophysical applications

We wish to point out an interesting effect associated with light emitted in the forward direction by a source circulating in an equatorial plane around a highly collapsed object or a black hole of mass M. The essential feature of the emission is that provided the radius a of the orbit in Schwarzschild's coordinates exceeds  $3GM/c^2$ , the light emitted is blueshifted when received by a distant observer. More specifically, if  $v_0$  is the frequency of light at emission and v the frequency at reception at a radial coordinate  $R \gg R_s = 2GM/c^2$ , we have

$$\frac{v}{v_0} = \frac{\sqrt{[(2-3\xi)(1-\xi)]}}{\sqrt{[2(1-\xi)]} - \sqrt{\xi}}$$
(1)

where

$$\xi = R_s/a \tag{2}$$

While  $v > v_0$  for all values of  $\xi < 2/3$ , it can be shown easily that as  $\xi \rightarrow 2/3$ , the blueshift diverges. Writing  $\xi = (2/3)(1-\epsilon)$ ,

$$v \simeq (2/3) v_0 \varepsilon^{-1/2} \text{ as } \varepsilon \rightarrow 0$$
 (3)

Thus very high blueshifts are obtained as the orbit of the particle tends to the so called unstable circular orbit. Qualitatively, we can understand the result in terms of the competition between the Doppler blueshift of forward light emission and the gravitational redshift due to the central mass. The former increases much more rapidly than the latter as  $\epsilon \rightarrow 0$ . We are interested here with orbits with very small  $\varepsilon$ , that is, orbits close to the unstable circular orbit.

The behaviour of matter revolving very close to such an orbit has been considered by several authors<sup>1-3</sup>. For example, an electron moving in a circular orbit can emit synchrotrontype radiation. It is also possible for gravitational radiation to be emitted by revolving matter. Such effects, however, have turned out to be very small even in the limiting case of  $\varepsilon \rightarrow 0$ . Here we suggest an alternative situation where the above mentioned result can play a significant part.

Before coming to the astrophysical setting consider what happens when we have matter moving in a thin ring round an object with mass M, with say  $\varepsilon_1 \le \varepsilon \le \varepsilon_2 \le 1$ . Suppose matter in this region has uniform distribution and is emitting light in a band of frequencies peaked round  $v_0$ , say. Then purely geometrical considerations and equation (3) lead to a reception spectrum at R of the form

$$F(v) = A(v_0) v^{-1}, v \ge v_0$$
 (4)

The function  $A(v_0)$  depends on the dimensions of the object and the emissivity of matter under consideration. The derivation of equations (3) and (4) is somewhat involved and will be published elsewhere, but the  $v^{-1}$  dependence of the spectrum purely on geometrical ground is noteworthy.

Turning now to the astrophysical considerations, it is well known that a highly collapsed object or a black hole tends to accrete matter. In general the infalling matter has some angular momentum so that it does not fall in radially. Instead, one can imagine matter making revolutions round the object several times before falling in. The unstable circular orbit is likely to play an interesting part in this process. The infalling matter goes round in orbits with small  $\varepsilon$  several times before getting sucked in or thrown out again<sup>1</sup>. So we may consider the thin ring discussed above as made up of transient matter.

The v-1 spectrum is common to many extragalactic sources of radiation. We have considered the continuum emission of QSOs whose spectrum can be approximated this way<sup>4</sup>. By considering thermal emission from hot gas in a narrow range of frequencies around  $v_0 \sim 3 \times 10^{14}$  Hz and taking the emissivity<sup>5</sup> as  $\sim 4.4 \times 10^{-24} n_e^2$  erg cm<sup>-3</sup> s<sup>-1</sup>, with  $n_e$  the electron density in cm<sup>-3</sup> we find that in order to explain the observations of a QSO like 3C273 in the optical and ultraviolet region on the basis of equation (4) we need a mass-distance relationship of the form

$$M/M_{\odot} \simeq 1.7 \times 10^{15} (R_{\rm mpc}/n_{\rm e})^{2/3}$$
 (5)

Here  $R_{mpc}$  is R expressed in Mpc. In a 'local hypothesis',  $R_{\rm mpc}$  may not exceed ~100 while  $n_{\rm e}$  can be taken as high as  $\sim 10^9$  to give a mass M of the order of that of a galaxy. Provided thermal emission is confined to relatively narrow band of frequencies at the source, the resulting spectrum at a large distance will show a  $v^{-1}$  dependence.

The light emitted in the forward direction makes several rounds of the object before reaching the distant receiver. As  $\varepsilon \rightarrow 0$ , the angle through which the light has turned can be shown to be

$$\varphi \sim \ln[(24 - 12\sqrt{3})/\epsilon]$$
 (6)

Thus for  $\varepsilon \sim 10^{-10}$  the light will go round nearly four times. J. V. N. thanks the Jawaharlal Nehru Fund for the award

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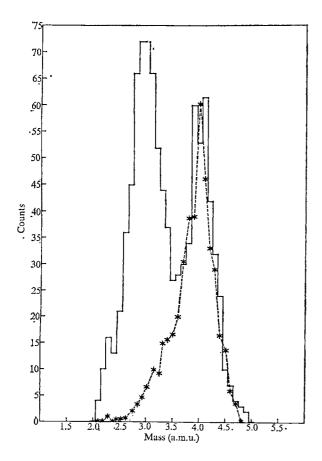
# Solar energetic particle event with $^{3}\text{He}/^{4}\text{He}>1$

OBSERVATIONS of  $\gamma$  rays and the short lived isotope <sup>3</sup>H provide evidence for the occurrence of nuclear reactions by high energy particles accelerated in solar flares1,2. Stable isotopes such as <sup>2</sup>H and <sup>3</sup>He should also be produced in these reactions. Solar <sup>3</sup>He particles were first detected by Hsieh and Simpson<sup>3</sup>. In the energy range 10 to 100 MeV per nucleon they obtained  $^3\mathrm{He}/^4\mathrm{He} = (2.1 \pm 0.4) \times 10^{-2}$  by summing over seven solar particle events. Garrard et al.4 and Anglin et al.5 have reported that 3He/4He was highly variable from event to event. (Table 1). In the '3He-rich events', 2H and 3H were not detected and the resulting upper limits were much less than expected from the theory of nuclear reactions6. These events were small, and the

Table 1 Relative abundance of He isotopes					
<sup>3</sup> He/ <sup>4</sup> He Solar particle events	<sup>4</sup> He/ <sup>1</sup> H Solar particle events	<sup>3</sup> He/ <sup>1</sup> H Solar particle events			
$1.52 \pm 0.1$ , May 28, 1969 (this paper) $0.54 \pm 0.09$ , July 30, 1970 (ref. 15) $0.26 \pm 0.08$ , October 14, 1969 (ref. 4) $0.077 \pm 0.02$ , November 2, 1969 (ref. 8) $0.021 \pm 0.004$ , average of seven flares in 1967 (ref. 3)	$0.40 \pm 0.04$ May 28, 1969 $\sim 10^{-2}$ , average of seven flares in 1967 (ref. 3) $\sim 1.7 \times 10^{-2}$ (ref. 16, average of seven large flares in solar cycle 20)	0.60 ± 0.05, May 28, 1969 ~ 0.09, July 30, 1970 ~ 0.005, October 14, 1969 ~ 0.0005, general range of occurrence in ordinary solar events			
Chromosphere $\leq (1.0 \pm 0.5) \times 10^{-2} \text{ (ref. 9)}$ Solar wind $4 \times 10^{-4} \text{ (ref. 11)}$ $\leq 4 \times 10^{-4} \text{ (ref. 10)}$	~ 1.4 × 10 <sup>-4</sup> , September 25, 1969 (Dietrich and Simpson, unpublished) Chromosphere and prominences ~ 0.06 (ref. 12) Solar wind ~ 0.045 (ref. 17)				

number of  ${}^{3}$ He particles observed was low ( $\sim$  70). This anomalous production of  ${}^{3}$ He should provide new insight into the acceleration and confinement process of energetic particles in solar flares.

Using the Goddard cosmic-ray telescopes of OGO-V (ref. 7) we detected an unusual solar event on May 28, 1969. The flare associated with the event occurred at 1248 local time and had importance 1B. The solar coordinates of the event are 10°N and 56°W with the associated McMath plage region 10109. About 600 ³He nuclei were detected in this event and  $^{3}\text{He}/^{4}\text{He} = 1.52 \pm 0.1$  in the energy range 4 to 80 meV per nucleon. This is the highest ratio reported so far for any solar event.



. Fig. 1 Mass histogram for He nuclei observed in the solar particle events on May 28 and November 2, 1969. Note dominance of <sup>3</sup>He in the May 28 event. The <sup>3</sup>He/<sup>4</sup>He ratio for the November 2 event is 0.08, in agreement with that reported by Dietrich<sup>8</sup>.

—, May 28 event; \*, November 2 event.

In Fig. 1 we give the mass histograms for He nuclei. The dotted curve represents the mass distribution (normalised at the peak of <sup>4</sup>He distribution) for another event on November 2, 1969. For this event we get <sup>3</sup>He/<sup>4</sup>He = 0.08, in excellent agreement with Dietrich<sup>8</sup>.

The ratio of hydrogen to helium for the event on May 28 is also highly unusual with  $^1H/(^3He+^4He)=1.00\pm0.05$  (Table 1).

The energy spectra of three components were all consistent with power laws in kinetic energy per nucleon. The exponents for  $^{1}$ H,  $^{3}$ He and  $^{4}$ He were  $-4.29 \pm 0.10$ ,  $-4.16 \pm 0.10$  and  $-4.04 \pm 0.11$ , respectively

The observed upper limit to  ${}^{3}\text{He}/{}^{4}\text{He}$  in the solar chromosphere was  $(1 \pm 0.5) \times 10^{-2}$  (ref. 9). In the solar wind  ${}^{3}\text{He}/{}^{4}\text{He} \sim 10^{-4}$  (refs 10 and 11). The solar particles have higher relative abundance of  ${}^{3}\text{He}$  in comparison with the solar chromosphere. The  ${}^{4}\text{He}/{}^{1}\text{H}$  ratio is also large compared to the observations on solar prominences 12. In Table 1, the isotopic abundances of He as obtained from several related studies are shown for comparison.

Ramaty and Kozlovsky<sup>13</sup> have proposed a theory dealing with the production of <sup>2</sup>H, <sup>3</sup>H and <sup>3</sup>He in solar events. They have taken into account effects of kinematics of the reactions and the possible anisotropy of solar beams to explain the anomalously small <sup>2</sup>H and <sup>3</sup>H abundances compared to <sup>3</sup>He. They find that for energetic protons and 4He nuclei incident on the solar atmosphere, 3He will be preferentially emitted in the direction opposite to that of the incident particle whereas <sup>2</sup>H and <sup>3</sup>H will tend to have the same direction. For the giant flare in August, 1972 (ref. 14), they arrive at about 2 gm cm<sup>-2</sup> to account for  $^{3}\text{He}/^{4}\text{He}$  of  $\sim 0.02$  observed in the event. They also relate the y-ray intensities observed by Chupp et al.7 with the energetic protons observed in the event. The large production of 3He according to Ramaty and Kozlovsky, occurs only at low energies (about 0.1 meV per nucleon) and so some post acceleration is necessary to account for the observed particles.

Using the same model to explain the very high 3He/4He ratio in the May 28 event requires the passage through 2.3 nucleon mean free paths or some 170 g cm<sup>-2</sup> of material for protons. This further requires that the ionisation energy loss be balanced by continuous acceleration which means that a 50 MeV per nucleon He nucleus would have to receive a total energy of ~ 12 GeV. This seems highly implausible. One possible clue may be provided by the simultaneous observation of the solar X rays and electrons. Here it is found that only  $10^{-2}$  to 10<sup>-3</sup> of the electrons escape with a large fraction incident on the lower corona which produces the X-ray emission. A similar effect may be occurring with the nuclear component though the rigidities of the nuclear particles are larger by factors > 10<sup>2</sup>. The other secondary products <sup>2</sup>H and <sup>3</sup>H are not seen in this event. Assuming that one nucleus of <sup>2</sup>H (or <sup>3</sup>H) was seen during the event, the upper limit for <sup>2</sup>H/<sup>3</sup>He (or <sup>3</sup>H/<sup>3</sup>He)

is  $\sim 1/600$ . Simultaneous  $\gamma$ -ray observations would be of great value in identifying the type of process and the time scale on which it operates. These were not available for this event. It is also possible that the solar material involved in these flares has an anomalous composition to start with.

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## Formaldehyde polymers in interstellar space

THE precise composition of interstellar dust is as yet unknown. Recent studies of the wavelength dependence of extinction and polarisation favour a composite model of three components: (1) elongated dielectric particles of radii  $\sim 10^{-5}$  cm, which would explain the data in the 1-0.3 µm spectral region; (2) almost spherical graphite particles of radii  $\lesssim 2 \times 10^{-6}$  cm, which would explain the near and middle ultraviolet data in 0.3  $\gtrsim \lambda \gtrsim$ 0.2 µm; and (3) small dielectric particles which contribute mainly to extinction and albedo at wavelengths,  $\lambda \leq 0.2 \,\mu\text{m}$ .

There is considerable evidence to support the view that silicate grains exist in circumstellar envelopes around certain cool stars1. An emission feature in the 8-12 µm waveband occurring in the spectra of many cool Mira-type stars has been identified with particles of a mineral-like composition. It is also widely believed that silicate dust is responsible for a major part of the observed interstellar extinction and polarisation at visual wavelengths, in other words, that silicates make up component (1) above. The evidence for this is not convincing and several strong counter indications already exist. Applications of the Kramers-Kronig relationship to the observed interstellar extinction curve suggest that greater relative abundances

of Mg, Si to H than are consistent with cosmic abundance ratios are necessary to explain the observed extinction2.3. This discrepancy could be as much as a factor 10. It seems therefore, that the only elements present in sufficient quantity in the interstellar medium to contribute to the observed visual extinction coefficient,  $\kappa_{\nu} \sim 2$  mag kpc<sup>-1</sup>, are C,O and N. The possibility of O in the form of H<sub>2</sub>O ice has been discussed for many years, although a 3.1 µm ice band was not detected in the spectra of several moderately reddened stars4,5.

I have argued (N.C.W., unpublished) that formaldehyde molecules which have been detected in over a hundred interstellar clouds could condense on silicate grains. Condensation of  $H_2CO$  on silicate grains with temperatures  $\lesssim 20$  K is attended by polymerisation into chains

resulting in the formation of a crystalline polymer known as polyoxymethylene (POM). The constituent elements of this material are sufficiently abundant to provide the main contribution to the mass density of interstellar dust. These particles would grow as long whiskers under interstellar conditions and possess the required dielectric properties to account for component (1) in the composite grain model. In common with many other polymers, POM is moderately refractory with a melting temperature in the range 450-500 K. Such grains could survive in H II regions and radiate at temperatures  $T_{\rm g}$ ≤ 500 K thus accounting for many galactic infrared sources.

The optical refractive index of epoxy formaldehyde resin, which is likely to be similar to that of POM, is n = 1.58 (ref. 6). A cylindrical particle of radius close to  $1.5 \times 10^{-5}$  cm, with this value of n, possesses extinction and polarisation properties which are consistent with interstellar data7.

The strongest evidence adduced in support of the view that silicate grains are mainly responsible for the general interstellar extinction is the appearance of an 8-12 µm emission feature in the infrared spectrum of the Trapezium nebula, as well as a similar extinction feature in the spectrum of the BN star in the Orion nebula<sup>1,8,8</sup>. Most other 10 µm features observed in stars are confined to strictly circumstellar grains and do not necessarily relate to the behaviour of interstellar grains.

By a remarkable coincidence, POM polymers also exhibit strong optical activity in the 8-12 µm waveband. Figure 1 shows the absorption spectrum of POM films studied by Tadokoro et al<sup>10</sup>, for polarised light. Although this feature is comprised of two main bands, as in the case of silicates, a distribution function in average chain length will mask much of the fine structure, as the precise positions of individual bands depend on this parameter in the crystalline powder.

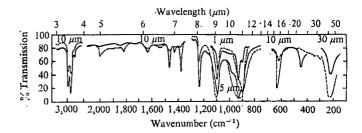


Fig. 1 Infrared transmission spectra for polarised light of POM-films of several thicknesses (1, 5, 10 and 30  $\mu$ m) for the various wavelength intervals as indicated in the insets. The solid curve refers to light with electric vector perpendicular to crystal axes; dashed curves for electric vector parallel to the crystal axes, (Differing thicknesses of film, 1, 5, 10 and 30  $\mu$ m, are used over various wavelength intervals. When this is noted, the 8-12  $\mu$ m bands are considerably stronger than others indicated in these data.)

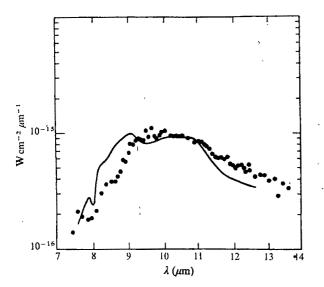


Fig. 2 The infrared emission (ω) from the Trapezium nebula compared with the theoretical flux from 445 K POM polymer grains (solid curve).

The average mass extinction coefficient at the centre of this band, estimated from the data of Tadokuro et al.10, is

$$\kappa_{10\mu m} \simeq 1.4 \times 10^3 \text{ cm}^2 \text{ g}^{-1}$$
 (1)

assuming a specific gravity s = 1.4 for POM. At the visual wavelength,  $\lambda_{\nu} = 5,470 \text{ Å}$ , the mass extinction coefficient for POM grains of radii,  $a = 1.5 \times 10^{-5}$  cm, is

$$\kappa_{\nu} \simeq (3Q_{\rm ext}/4as) \simeq 7.1 \times 10^4 \,\rm cm^2 \, g^{-1}$$
 (2)

Equations (1) and (2) give  $\kappa_{\nu}/\kappa_{10\mu m} \simeq 51$ . The corresponding ratio inferred from the astronomical data for extinction in the BN star is very similar  $(\kappa_{\nu}/\kappa_{10\mu\,\mathrm{m}})_{\mathrm{BN}} \simeq 50$  (ref. 1). Figure 1 shows that interstellar POM grains could give rise to somewhat weaker, yet probably detectable, features at  $\lambda \simeq 3 \,\mu m$  and  $\lambda \simeq 16-23 \,\mu m$ . The latter feature imparts a further ambiguity to the identification of silicate grains, whilst the former may be confused with the 3.1 µm band of H<sub>2</sub>O ice.

Absorbance data for heated POM films10 may be used to compute an approximate emission spectrum of small POM grains. The absorption efficiency of small particles, irrespective of shape, is proportional to the absorbance  $\gamma_{abs}$ , provided the refractive index n is close to 1 and the absorptive index kremains small throughout the band. Assuming these conditions to be fulfilled the emission spectrum of POM grains may be calculated by

$$F_{\lambda} \propto \gamma_{abs} B_{\lambda}(T_{g})$$

where  $B_{\lambda}(T_{\rm g})$  is the Planck function,  $T_{\rm g}$  is the grain temperature. The emission spectrum of the Trapezium nebula is shown in Fig. 2 together with a normalised emission spectrum for POM grains heated to 445 K. The agreement is as good as, if not better than, for any silicate model. POM grains are thus able to explain all available interstellar extinction and emission data.

In view of the spectral identifications presented here POM grains must clearly be regarded as a strong candidate for the main component of interstellar dust. Such grains could contribute a significantly higher mass density than silicate grains. More detailed measurements of the complex refractive index of POM as a function of wavelength and of temperature are required before further comparisons with astronomical data can be made.

Our provisional conclusion is that interstellar grains consist of a mixture of small graphite particles and silicate particles coated with POM mantles. The graphite and silicate particles

are ejected from cool giant stars; silicate grains have low enough temperatures under interstellar conditions to accrete POM polymer mantles.

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# Pacific equatorial pressure gradient and Indian monsoon rainfall

SEA surface temperature (SST) anomalies are known to affect atmospheric flow patterns considerably. The Indian monsoon circulation is induced by the thermal contrast between the Asian continent and the Pacific and Indian oceans and by the orography of the Himalayas, so large scale SST anomalies are likely to affect the monsoon circulation, but after some time lag.

In recent years SST anomalies over the Pacific have been associated with Walker's Southern Oscillation1,2. We find that the factor (Mataveri-Darwin) pressure difference used by Ouinn and Burt as a measure of the strength of the Southern Oscillation is also quite useful in predicting monsoon rainfall.

Figure 1 shows the relationship between the seasonal (June-September) monsoon rainfall of north-west India and the mean

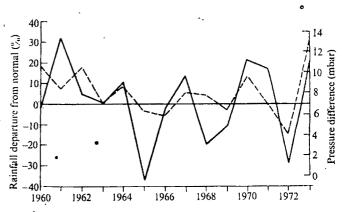


Fig. 1 Relationship between rainfall and pressure gradient. -Departure from normal (%) for seasonal (June-September) rainfall in north-west India. — —, Mean Pacific pressure gradient (Mataveri-Darwin) during April and May.

Pacific pressure gradient (Mataveri-Darwin) during April and May, from 1960 to 1973. There is some correspondence between the two curves (except for 1968) and the large contrast between the drought year of 1972 and the good monsoon year of 1973 is evident in both cases. In the drought years of 1972, 1965 and 1966, this average pressure difference fell below 7 mbar. The area of that part of the pressure gradient curve below 8 mbar, as used by Quinn and Burt, seems to be a good indicator. In 1972, an El Nino year, the pressure difference fell to as low as 0.5 mbar in May, whereas it was 7.6 mbar in May 1973. Pacific typhoon activity was also much above normal in 1972 but below normal in 1973.

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## New pole for early opening of South Atlantic

Using the trends of equatorial, marginal fracture ridges, Le Pichon and Hayes<sup>1</sup> proposed an early phase for the opening of the South Atlantic, with a pole of rotation at 21.5°N, 14°W with respect to Africa. Francheteau and Le Pichon<sup>2</sup> tested this plate tectonic model with the whole South Atlantic and assumed that there is a relationship between continental margin offsets, the subsidence of coastal basins, and adjacent marginal fracture zones. We have studied extensions of fracture zones

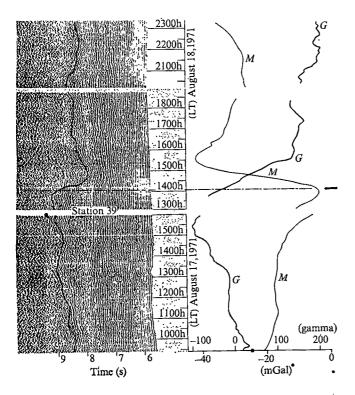


Fig. 1 Seismic profile across the Chain Fracture Zone south of Togo (location on Fig. 2). Note the difference in level of the acoustical basement under a thick sedimentary cover. The free air gravity anomaly is of the order of 40 mGal. The arrow indicates the theoretical location used for the determination of the trend of the fracture zone. M, Magnetic curve; G, gravity

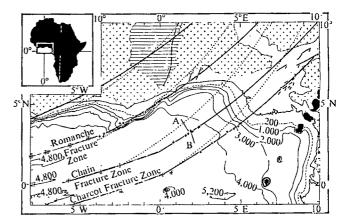


Fig. 2 Eastern Gulf of Guinea. Bathymetric contours (m) from E. Uchupi (unpublished); geological sketch from the geological map of Africa19. Crosses, cratonic area; hatched, Palaeozoic platform; dotted, Mesozoic to Recent basins; black area, Cameroon volcanic line. Heavy crosses, positions of geophysical features (from seismic, gravity, or magnetic surveys) used for computation of the new early pole of opening. Heavy black lines, portion of small circles about the new early pole along the Saint-Paul, Romanche, Chain and Charcot Fracture zones. Note the good agreement between the extension of the two last fracture zones and the main geological trends in Nigeria. Dotted lines, portions of small circles about the previously proposed pole of opening. Note the discrepancies with the geological trends in the Benoue Trough area. Double lines, posterior fracture zone trends. A-B indicates profile of Fig. 1.

in the Gulf of Guinea and adjacent continental margins and have determined a new early opening pole that differs markedly from the one previously determined.

Thanks to deeper penetration seismic techniques, extensions of the well known equatorial fracture zones3 have been traced into the Gulf of Guinea<sup>4-7</sup> under a thick sedimentary cover mostly related to the Niger Delta. Figure 1 shows a seismic section across the extension of the Chain Fracture zone. It has been proposed8 that the position of the inflexion point on the regional free-air anomaly curve corresponds to the main changes in the average basement level; consequently, seismic and gravity criteria have been used both together and separately to locate the position of the fracture zone. A structural trend called the Charcot Fracture Zone7,9,10 has been discovered just south-west of this delta (Fig. 1).

We have re-interpreted these results to obtain a better knowledge of the early opening phase of the Gulf of Guinea and consequently of the South Atlantic. The positions determined of the main geophysical features considered here are shown on Fig. 1. The Saint Paul Fracture Zone itself has not been taken into account, mainly because of its complex structural pattern near the Liberian continental margin<sup>11</sup>. The trend of the Chain Fracture Zone previously inferred from bathymetric data1,5 is not compatible with our data (Figs 1 and 2). Parameters of our new pole are 32°N, 20°W (with a standard deviation of 6 km).

The great circle defined by the two poles of rotation (21.5°N, 14°W and 32°N, 20°W) cuts across the Liberian continental margin, the Walvis Ridge and the Aghulas Fracture Zone<sup>12,13</sup> along a line close to the boundary that existed between Africa and South America before the opening of the ocean. Consequently, small circles about the two poles of rotation are tangential to each other, especially in the area where the great circle lies close to preopening boundaries. They diverge as the distance from the great circle increases, as in the eastern part of the Gulf of Guinea. Moreover, this effect decreases as the distance from the pole increases.

Figure 2 shows the small circles about each pole of rotation in the Gulf of Guinea. The direction of early opening which we propose fits well with the Liberian-Ivory Coast continental slope and with the limit of the Togo-Dahomev Basin. It also

agrees much better than did the previously proposed direction with the broad geological structure in Nigeria. It allows the extension of the Chain Fracture Zone to be related more easily with the northern hinge line of the Niger Delta<sup>14</sup> and the northern limit of the Benoue Trough<sup>15</sup>. The Charcot Fracture Zone seems to correspond well with the complex Abakiliki area in southern Nigeria16.

Figure 3a shows the good agreement between small circles about the new pole (32°N, 20°W), and fracture trends in the vicinity of the Liberian continental margin<sup>11</sup>. Figure 3b and crepresent, respectively, the eastermost section of the Walvis Ridge<sup>17</sup> and the Agulhas Fracture Zone<sup>12,13,18</sup> on which a computed theoretical direction from the new pole has been superimposed. As expected from the orientation of the great

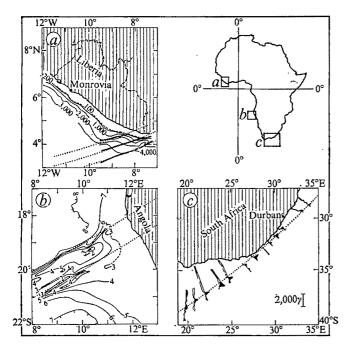


Fig. 3 Comparison between some western South Atlantic marginal structures and the new theoretical direction (small circles about a pole at 32°N, 20°W). a, Liberian continental margins (depth contours in metres) and the Saint-Paul, Cape Palmas, Grand Cass fracture zones<sup>10</sup>; b, depth (in seconds) of easternmost Walvis Ridge basement<sup>6</sup>; magnetic anomalies in the Agulhas Fracture Zone.

circle and the distance from the poles, the agreement in both cases is good. It is interesting to note that the easternmost Agulhas Fracture Zone differs somewhat from the theoretical direction but it should be specified that this fracture zone has for the most part been inferred from magnetic anomalies13. Those anomalies could be related partly to the continentaloceanic crust boundary instead of being representative of only the fracture zone itself.

Although in many cases this newly established evidence will have to be taken into account in any detailed reconstruction of the opening of the Atlantic, the conclusions of Francheteau and Le Pichon<sup>2</sup> still seem to be valid.

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# Magnetic polarity of **Explorer Ridge basalts**

THE Vine-Matthews hypothesis1 connects geomagnetic polarity reversals with marine magnetic anomalies through the process of seafloor spreading. This hypothesis requires a one-to-one correlation between the sign of a given marine magnetic anomaly and the magnetic polarity of underlying oceanic basalt. Because properly oriented samples are lacking, however, this feature remains virtually untested in spite of its crucial significance.

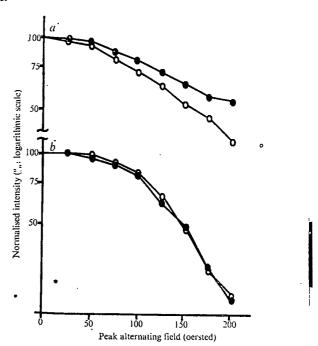


Fig. 1 Demagnetisation curves interpreted as: a, reversed polarity (specimen 717002A); b, normal polarity (specimen 701520A). The size of the symbols represents experimental uncertainty. ●, NRM; ○, ARM acquired in a bias field of 0.58 oersted and a peak alternating field of 1,800 oersted.

	Table 1 Overhead magnetic anomalies and inferred polarities									
Dredge site	Location*	Overhead anomaly†	Age (Myr)‡	Specimen	$\Delta_{0}$	$\Delta_{25}$	$\Delta_{50}$	$\Delta_{75}$	$\Delta_{100}$ §	Inferred polarity
7004	50° 14.1′N 130° 15.4′W	+200γ	<1	700407A 700418A 700425A 700427A 700434A 700465A	+1.3 +2.6 +4.5 +3.0 +1.5 +6.1	+0.4 $-0.6$ $+4.3$ $+1.9$ $+1.7$ $+7.0$	-0.8 $-0.4$ $+1.8$ $+1.9$ $+1.5$ $+7.6$	-0.5 $+2.4$ $+1.3$ $+1.3$ $+8.2$	$ \begin{array}{r} -1.3 \\ -0.1 \\ +2.5 \\ +2.7 \\ +0.3 \\ +8.0 \end{array} $	N N (N?) N N R
7015	49° 45.3′N 130° 17.3′W	+300γ to +400γ	<1	700482A 701504A 701520A 701522A 701523A 701525A 701533A 701534A	+1.3 $+0.9$ $-0.6$ $-1.3$ $-2.0$ $-0.7$ $-1.1$ $+0.5$	-3.0 -1.2 -1.2 -1.8 -1.9 -1.0 -1.2	$ \begin{array}{r} -1.5 \\ -2.6 \\ -0.3 \\ +0.6 \\ -2.2 \\ -2.3 \\ -1.0 \\ -1.0 \end{array} $	$     \begin{array}{r}       -2.8 \\       +1.0 \\       -0.7 \\       -0.8 \\       -0.4 \\       +1.1 \\       -1.3 \\       -0.8 \\    \end{array} $	$\begin{array}{c} -1.2 \\ +0.6 \\ -0.1 \\ -0.2 \\ +0.7 \\ -2.3 \\ -1.2 \\ -1.0 \end{array}$	מממממממממ
7017	50° 03.8′N 129° 41.8′W	+300γ to +600γ	<1	701701A	+0.4	-0.1	-0.9	+5.3	+5.2	(R?)
7170	49° 07.9′N 130° 34.8′W	—300γ —300γ	1 to 2	717001A 717002A 717004A 717008A 717009A	+7.3 +9.0 +8.0 +6.4 +8.0	+7.6 +9.1 +8.1 +5.7 +7.1	+7.4 +9.6 +7.8 +6.8 +7.5	+7.3 +8.5 +6.8 +7.2 +7.4	+9.1 +7.4 +5.9 +9.8 +6.3	R R R R
7192	50° 12.1′N 129° 52.6′W	—500γ	1 to 2	719202A 719203A 719204A 719207A 719208A 719210A	+5.4 +7.6 +4.3 0 +0.6 +0.8	+2.3 +2.9 +6.9 -1.9 -1.5 -0.1	+6.3 $+4.3$ $+13.3$ $-0.5$ $-1.0$ $+2.1$	+10.2 +3.4 +19.7 -4.3 -3.6 +3.7	+12.1 +13.1 +11.6 -0.8 +8.5	R R R R N N (R?)

\* Best satellite Navigator fix for terminus of dredge haul.

Under certain circumstances<sup>2,3</sup>, the polarity of the original remanence of unoriented dredged samples can be recovered. It is essential for the success of the method that there be present secondary magnetic components in the samples resulting from magnetic viscosity of the oceanic basalts in the presence of the recent geomagnetic field, and these can be expected to parallel the primary remanence of normal polarity basalts and to oppose that of reversed material, provided that the rocks have remained in situ. Under appropriate conditions this may lead to a detectable difference between the alternating field demagnetisation curves of natural remanence (NRM) and laboratory induced remanence; anhysteretic remanence (ARM) is used to avoid problems associated with reheating. Irving<sup>2</sup> has suggested that a normalised NRM curve lying significantly above the corresponding ARM curve is indicative of reversed primary remanence.

This method (and slight variations of it) has been applied to samples from the Mid-Atlantic Ridge. Agreement has been found<sup>4,5</sup> between inferred polarity and overhead anomaly in 20 out of 41 specimens. A positive correlation has been found in 16 out of 20 specimens dredged from the Juan de Fuca Ridge<sup>6</sup>.

We are completing a study of the magnetic properties of a suite of basalt samples dredged from the Explorer Ridge which represents an extension of the Juan de Fuca Ridge north of the Sovanco Fracture Zone<sup>7,8</sup>. The details will appear elsewhere; here, we report a substantial correspondence between inferred magnetic polarities and those predicted by the Vine-Matthews hypothesis. Twenty-six basalt samples(one specimen per sample) from five separate dredge hauls have been investigated. The locations of the five hauls (Table 1) lie within the central positive magnetic anomaly and the adjacent negative anomaly<sup>9,10</sup>. They are within 40 km of active spreading centres and range in age from 0-2 Myr.

We have found that the region between 0 and 200 oersted is

the most useful part of the coercivity spectrum as the ARMs become weak and generally unstable at higher peak fields. Results indicating clear cases of each polarity are given as examples (Fig. 1). The 26 specimens have been classified according to the relative hardness of their ARM and NRM demagnetisation curves.

One must seek assurance that any apparent differences between NRM and ARM coercivity spectra are significant compared with experimental uncertainty. For strongly magnetised rocks, this is no better than about 2%. To be conservative we have sought an average difference of 4% to establish firmly any divergence of the two curves, although Table 1 indicates that this choice is not too critical for most of the samples. In addition, the alternating field (a.f.) treatment to which the two curves should be normalised must be investigated fully. This second point arises because of the magnetic noise generated by additional short-lived components of magnetisation acquired between departure from outcrop and measurement<sup>2</sup> These unwanted viscous components can be effectively filtered off by normalising to successively higher fields<sup>2</sup>. To test the difference, or lack of difference between NRM and ARM, and also to investigate the effect of different normalising fields, the mean difference,  $\Delta_h$  (ref. 5) between the two spectra at the various treatments has been calculated. The values of  $\Delta$  at different normalising fields, h, are given in Table 1. As the appropriate normalising field is not known in advance we first of all classified those cases in which all normalisations yielded the same inferred polarity. Thus, 14 specimens could be regarded as definitely normal and 7 specimens as definitely reversed. A further two specimens (719202A and 719203A) showed generally large positive differences with isolated dips below +4%: these are very probably reversed. Of the remaining three specimens, 700425A is classified as normal as its  $\Delta$  values exceeds +4%(by very small margins) only at 0 and 25 oersted. Specimens

<sup>†</sup> From data of Raff and Mason<sup>13</sup>; range given where hauls crossed steep gradients. ‡ From interpretations of magnetic anomalies by Vine<sup>9</sup> and Silver<sup>10</sup>.

 $<sup>\</sup>delta \Delta h = \frac{100}{N} \sum \delta_i$ , where h is the normalising field; N is the number of demagnetising steps between h and 200 oersted, and  $\delta_i = \frac{100}{N} \sum \delta_i$ , where h is the normalising field; N is the number of demagnetising steps between h and 200 oersted, and  $\delta_i = \frac{100}{N} \sum \delta_i$ , where h is the normalising field; N is the number of demagnetising steps between h and 200 oersted, and  $\delta_i = \frac{100}{N} \sum \delta_i$ , where h is the normalising field; N is the number of demagnetising steps between h and 200 oersted, and  $\delta_i = \frac{100}{N} \sum \delta_i$ , where h is the normalising field; N is the number of demagnetising steps between h and 200 oersted, and  $\delta_i = \frac{100}{N} \sum \delta_i$ , where h is the normalising field; N is the number of demagnetising steps between h and 200 oersted, and  $\delta_i = \frac{100}{N} \sum \delta_i$ , where h is the normalising field; N is the number of demagnetising steps between h and 200 oersted, and  $\delta_i = \frac{100}{N} \sum_i \delta_i$ , where h is the normalising field; (normalised NRM-normalised ARM) at field i.

<sup>¶</sup> Demagnetising step inadverdently omitted.

Three cases, discussed in the text, have tentative polarity assignments only and are designated by parentheses.

701701A and 719210A are tentatively classified as reversed because their demagnetisation curves begin to diverge significantly at higher fields.

We therefore feel that 23 of the 26 assigned polarities are quite firmly established; the remaining three are rather tentative. The significant result is that a total of 20 out of the 23 specimens (or 22 out of 26 if the dubious assignments are included) yield polarities in agreement with the overhead anomaly (Table 1), lending strong support to the Vine-Matthews hypothesis. The few cases of disagreement give no cause for alarm. Indeed, a small percentage of samples of opposite polarity are expected from gravity-transported boulders and from contamination because of the finite width of intrusive activity at accreting plate boundaries11,12.

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# 3,800-Myr granitic gneiss in south-western Minnesota

We have previously arrived at an age of 3,550 Myr for granitic<sup>1</sup> gneiss in the vicinities of Morton and Montevideo in the Minnesota River valley, south-western Minnesota. We now report new Rb-Sr analyses (Table 1) and an age of 3,800 Myr for the fine grained foliated phase of the Montevideo Gneiss of Lund<sup>2</sup>. The rocks have undergone a complex history of metamorphism which remains to be deciphered, but the age determinations reveal that the geological mapping and previous interpretations did not provide a proper basis for sampling.

Table 1 Analytical data						
Sample no.	p.p.m	. (weight)	Ratio (atomic)			
•	Rb Î	Sr	87Rb/86Sr	87Sr/86Sr		
KA-209	79.4	204.1	1.132	0.7619		
KA-54	75.0	270.1	0.8066	0.7455		
605	80.0	287.8	0.8076	0.7434		
606	89.1	256,8	1.009	0.7544		
607	41.5	378.7	0.3183	0.7206		
608	92.7	307.2	0.8769	0.7472		
609	71.7	339.0	0.6136	0.7324		
	, ,	557.0	0.0150	0.7524		

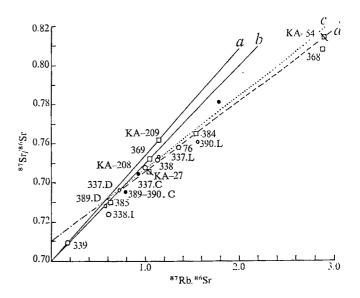


Fig. 1 Rb-Sr isochron diagram of whole-rock (squares) and Kfeldspar samples (crossed squares) from the Montevideo Gneiss and whole-rock samples (large open circles) from the Morton Gneiss (small open circles, samples in composites; small closed circles, composites). (From Goldich et al.\(^1\).) a, 3,800 Myr (0.700); b, 3,550 Myr; c, 2,650 Myr (0.710); d, 2,550 Myr.

Our original age of 3,550 Myr. ( $t_{\downarrow} = 50 \times 10^9 \text{ yr}$ ) was based on our interpretation of discordant U-Pb zircon ages and a Rb-Sr age computed for three whole-rock samples (Fig. 1, numbers KA-209, 369, 385). The 2,650-Myr isochron (Fig. 1) represents a time of high grade (granulite facies) metamorphism and is based on Rb-Sr rock-mineral isochrons and on nearly concordant U-Pb zircon ages1. The 2,550-Myr isochron approximates the time of uncovering and of stabilisation of the Rb-Sr system in the K-feldspar. A line through the points for sample KA-209 from the Montevideo Gneiss and sample 339 from the Morton Gneiss gives an age of 3,800 Myr with an initial 87Sr/86Sr of 0.700 (Fig. 1).

We did not previously attach any significance to this line because only two data points were involved. Of the six new samples from the Montevideo Gneiss, however, four lie on the 3,800-Myr isochron and two above this line (Fig. 2). The difference between the measured ratios for samples 607 and KA-54 and the other ratios are real and outside analytical error  $(\pm 1\% \text{ for } {}^{87}\text{Rb}/{}^{86}\text{Sr} \text{ and } \pm 0.0002 \text{ for } {}^{87}\text{Sr}/{}^{86}\text{Sr}); \text{ hence,}$ inclusion of these samples in an isochron plot is not desirable and some sort of geological explanation is required. The most obvious one is that during the complex history of metamorphism and igneous activity some metasomatic action was involved; for example, Rb and Sr moved among the rock units creating an open system. A gain in Rb or loss in radiogenic 87Sr would lower the ages and explain the scatter of the whole-rock and K-feldspar points in Fig. 1. The apparent ages of the gneiss samples (Fig. 2) therefore must be considered minimum values. If it is argued that the apparent ages are too old, the explanation must invoke either loss of Rb or gain of radiogenic 87Sr. We favour such an explanation with a net gain of 87Sr for samples KA-54 and 607.

A least squares calculation, excluding KA-54, 607 and 339, gives an age of 3,950  $\pm$  70 Myr (2 $\sigma$ ) with an initial  $^{87}$ Sr/ $^{86}$ Sr ratio of 0.698  $\pm$  0.004, exceptionably low for terrestrial rocks. We, therefore, prefer to include sample 339 from the Morton Gneiss as a guide to the initial ratio of 0.700 and an age of 3,800 Myr.

The history of the Precambrian rocks in the Minnesota River valley has some marked resemblances to the events that have been dated3 in the ancient rocks of West Greenland. As in Greenland, the oldest rocks in south-western Minnesota

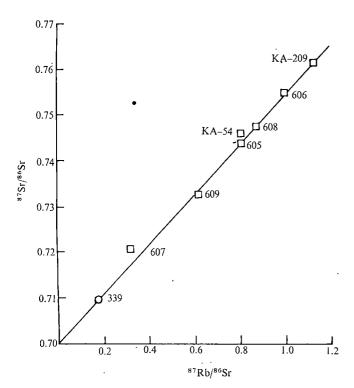


Fig. 2 Whole-rock samples from the foliated phase of the Montevideo Gneiss ([]). O, Morton Gneiss—3,800 Myr (0,700).

are gneisses ranging in composition from tonalite to granodiorite and quartz monzonite. These were intruded by granite and pegmatite of one or more periods of magmatic activity. Preliminary results indicate an age of approximately 3,000 Myr for some of the massive layers of granite which were formerly considered an original component of the Montevideo Gneiss1. Foliated and massive phases were folded together during the high-grade metamorphic event 2,650 Myr ago, at which time large volumes of granite were emplaced in south-western Minnesota. Basaltic dykes were intruded in several periods, the older ones being involved in the 2,650-Myr metamorphism and predating the granitic activity of approximately 3,000 Myr ago. Some dykes are much younger and were intruded before the emplacement of granite 1,850 Myr ago.

Work is in progress on U-Pb and Rb-Sr dating of the old gneissic rocks and of the younger rock units. The work at Northern Illinois University was supported by a grant from the National Science Foundation. We thank G. R. Himmelberg and R. L. Bauer of the University of Missouri for help in the sampling.

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### Anomalies in particle shape during seeded growth of polystyrene latices

We have observed unusual changes in latex particle shape during the 'seeded' growth of polystyrene latices in emulsifierfree systems (Fig. 1a). To our knowledge this has not previously been recorded.

Polystyrene latices may be grown in the absence of emulsifier1,2 (Table 1); however, the ultimate size of the latex produced is limited. In an effort to obtain larger particles, using an emulsifier-free technique, we used a two-stage process whereby a latex of selected size was utilised as the 'seed' to grow larger particles.

Table 1 Normal growth process						
Time after initiation (min)	Size (nm)	Standard deviation (nm)	B <sub>1</sub>	$B_2$		
1 5 15 30 60 90 120 175 275 302 330	20.3 37.5 85.3 101.4 145 176 204 244 308 327 347	4.2 2.7 3.7 2.9 3.3 2.0 4.2 2.0 3.5 4.0	1.69 0.03 0 0.05 0.11 0.61 0.14 0.01 0.09 0.09	6.02 3.04 2.57 2.33 2.93 2.45 3.49 2.29 2.92 1.83 2.35		
330 1,257	. 435	3.7 4.0	0 0.45	2.35 6.59		

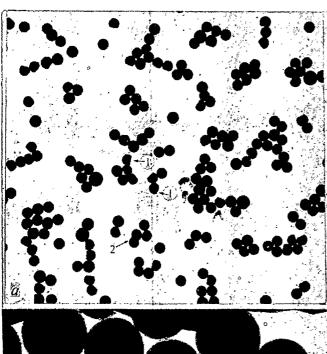
 $B_1$  and  $B_2$  describe the skewness and kurtosis of the particle size distribution and should be 0 and 3 for normal distribution3.

In a typical seeded growth process, a sample (50 g of 51% w/w polystyrene latex of diameter 520  $\pm$  11 nm) was agitated for 24 h at 70  $\pm$  0.2° C with 25 g of styrene monomer, 54 g distilled water and 0.02079 g potassium persulphate free radical initiator dissolved in 19.5 g distilled water. Samples were taken at intervals and sizes determined by means of electron microscopy (Table 2). The latex particles increased in size, through stages which were themselves highly monodisperse, to a diameter of 606  $\pm$  5 nm, an increase of approximately 20%.

Two hours after the start of the experiment, new polymer particles of diameter about 25 nm appeared. These particles formed agglomerates, which in turn attached themselves to the original polymer seed spheres. Since no small particles were present at the end of the reaction, we inferred that they had contributed to the growth of the 'seed'. After 4 h, incomplete spheres, eroded to varying degrees, were observed and from

	Table 2	Seeded growt	h process	,
Time after initiation (min)	Size (nm)	Standard deviation (nm)	$B_1$	$B_2$
0 22 58 133 182 244 301 362 1,440	520 (520) 525 526 522 (528) 542 (531) 542 (540) 562 589 625 (606)	11.0 (11.0) 5.0 6.0 5.0 (4.0) 7.0 (5.0) 4.0 (9.0) 8.0 30.0 6.0 (5.0)	0.05 (0.05) 0.05 0.16 0 (0.22) 6.02 (0.54) 0.62 (1.29) 0.09 3.41 0.33 (0.01)	4.00 (4.00) 4.08 3.75 1.99 (2.46) 2.42 (2.56) 3.18 (4.09) 2.79 7.72 5.96 (2.07)

Values in parentheses refer to process where all reactants were added and polymerisation begun immediately. See also legend for Table 1.



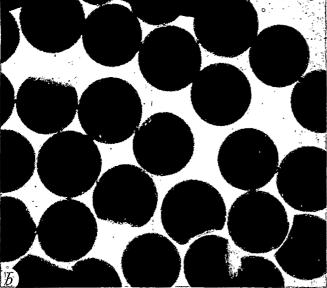


Fig. 1 a, Eroded polystyrene latex particles produced during an emulsifier-free seeded growth process. Seed latex  $520 \pm 11$  nm 362 min after initiation of reaction, size  $589 \pm 30$  nm. b, Eroded particles at high accelerating voltage (100 KV) to reveal internal detail.

measurements we concluded that, in some cases, erosion must have involved some of the original 'seed'. This dissolution of hardened polymer spheres is difficult to explain. Erosion increased until, after 6 h, the phenomenon was most pronounced with large proportions of some spheres eroded away, for example Fig. 1a, arrow 1. At the end of the polymerisation (24 h), however, the erosion was no longer seen, the spheres having apparently reformed.

Detailed examination in the electron microscope at higher accelerating voltage (Fig. 1b) revealed the presence of eroded particles with varying orientations and it is possible that the majority of particles was affected. The formation of incomplete latex spheres did not seem to be a consequence of specimen preparation and subsequent electron microscopy since it was not recorded during normal growth processes.

In order to study the possible effect of the monomer on swelling and erosion of the polymer particles, a sample of 'seed' latex was left in contact with monomer overnight at room temperature before the initiator was added and the 24 h polymerisation carried out. A similar abnormal growth process was observed. (A sample of this particular grown latex (625  $\pm$  6 nm) was stored for 5 months at room temperature after which no further erosion, caused by storage in unreacted monomer, was observed.)

We note that in some cases the incomplete spheres appeared to originate from groups of two or more, where clear interlocking occurred (Fig. 1a, arrow 2). Examination of numerous particles, however, failed to reveal any spheres with two or more eroded portions. Such particles might be expected as the result of the break-up of fused spheres.

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### Oriented glass ceramic fibre

Longitudinal velocity gradients in a flowing system have a powerful orienting influence on macromolecules and have been used to align polyethylene chains immediately before crystallisation<sup>1</sup> and from the melt<sup>2</sup>. Corning 119 SCR is a glass which crystallises to a glass ceramic containing fluorophlogopite (mica) as the principal crystalline phase. By subjecting it to pure extensional flow during crystallisation, strict alignment of the fluorophlogopite crystals has been achieved. Once produced, the alignment is maintained during subsequent drawing down to fibre diameters of less than 100 µm.

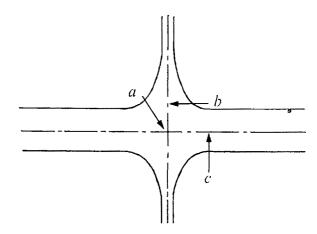


Fig. 1 Flow produced by impinging jets. a, Centre of symmetry; b, symmetry plane; c, symmetry axis.

A large strain rate, free from the complicating effects of rotation, exists along the axis (and in the plane of symmetry) of two opposed fluid jets meeting as shown in Fig. 1 (refs 1 and 3). Along the axis, the fluid velocity is zero at the centre of symmetry, increasing to a velocity  $\nu$ —equal to that for unrestricted flow—at a distance of approximately d/2, where d is the bore diameter

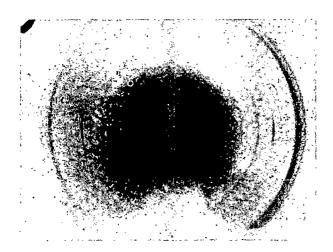


Fig. 2 X-ray pattern from an extruded rod. The rod axis is

of the jets. The corresponding strain rate is  $\varepsilon \sim -2\nu/d$ ; for example,  $2 \times 10^3$  s<sup>-1</sup> for v = 1 m s<sup>-1</sup> and d = 1 mm.

The same kind of flow field is obtained by extruding fluid through opposed dies; v is then the speed of the fluid leaving the dies and is determined by the speed of the extrusion ram and the bore diameters of the container and dies.

The extrusion press for this experiment consists of a stainless steel container placed inside the coil of a high frequency induction furnace and between the cross head and base plate of a tensile testing machine. Lowering the cross head pushes a pair of rams into two vertical channels which are interconnected by a

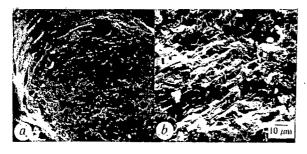


Fig. 3 Scanning electron micrographs of fracture surfaces. a, Normal to the axis of a 150  $\mu$ m diameter fibre drawn from a 2 mm diameter extruded rod; b, oblique to the axis of an extruded rod subsequently fully crystallised by annealing.

horizontal channel across the middle of the container. All three channels are filled with glass; the dies are oriented vertically and countersunk into the horizontal channel. Both the radial velocity gradient of the fluid entering the dies and the shearing flow of the fluid within the dies are also expected to have an orienting effect on the crystal nuclei.

Figure 2 shows the Laue transmission X-ray diffraction pattern from a rod extruded at the crystallisation temperature (950 °C) using an interdie strain rate of 165 s<sup>-1</sup>. Fluorophlogopite is hexagonal4 and the basal plane reflections 0003 and 0006 each take the form of a pair of arcs parallel to the rod axis. All other reflections occur as Debye-Scherrer rings because the crystals are oriented randomly about the hexad axis. Identical patterns have been obtained from rods extruded and then drawn down to diameters of 150 µm and 75 µm.

The alignment of crystals can be observed directly by scanning electron microscopy of fracture surfaces (Fig. 3a). The density of crystals is uniform except very near the fibre surface where it falls to almost zero. In drawn samples, the surface

contains a high density of shrinkage hollows. Completion of the crystallisation treatment, by annealing at 950° C, produces some growth of the oriented crystals plus growth of new and, therefore, unoriented nuclei. This second generation of crystals is evident in Fig. 3b.

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## The direct measurement of sulphur deposition on bare soil

THREE one-thousandth acre gauges, respectively 20, 40 and 60 inches deep were made at Rothamsted Experimental Station in 1870 by Lawes and Gilbert<sup>1</sup>. Since 1870 the soil in the drainage gauges has not received any fertiliser and has not been cropped. Amounts of nitrogen mineralised annually2 have fallen to about 7 kg ha<sup>-1</sup> and from this we have calculated that sulphur mineralised is about 1 kg ha-1 annually. Chemical analysis showed that the drainage water contains twice as much sulphur as is deposited in rain. A direct measurement of the dry deposition of sulphur was made to see if this satisfactorily accounted for the discrepancy.

Two grams of air-dried sieved soil (0.5 mm), with a total sulphur content of 9.2 μg S g<sup>-1</sup>, were exposed in Petri dishes inside a standard Stevenson meteorological screen at three sites, Rothamsted (Herts.), Woburn (Beds.) and Saxmundham (Suffolk), in mid-February 1974 for 57 to 77 d. Sulphur deposition rates were calculated from the increases in total sulphur content after exposure (Table 1). Air sulphur concentrations were measured at Woburn Experimental Station and Framlingham (near Saxmundham) and identical deposition velocities (Vg) calculated for both. Since, at this time<sup>3</sup>, the measured deposition rates would be close to the annual means, we have calculated depositions (kg S ha<sup>-1</sup>) of 18.1 (Saxmundham), 27.5 (Rothamsted) and 65 (Woburn) annually. An independent check on the Rothamsted figure (27.5 kg S ha<sup>-1</sup>) was made from the analysis of drainage water from the 20-inch deep, one-

Table 1 The period of exposure, increase and deposition rate of sulphur to bare soil at three sites and the air sulphur concentration and deposition velocity for two of the sites

	Saxmundham*	Rothamsted	Woburn
Period of exposure (d)	77	60	57
S increase, g cm <sup>-2</sup> S deposition	$38.2 \times 10^{-6}$	$45.2 \times 10^{-6}$	$101.6 \times 10^{-6}$
rate, g cm <sup>-2</sup> s <sup>-1</sup> S air concentration	5.74 × 10 <sup>-12</sup>	$8.72 \times 10^{-12}$	$20.63 \times 10^{-12}$
g m <sup>-3</sup> Deposition	$13.5 \times 10^{-6}$	-†	$47.8 \times 10^{-6}$
velocity (Vg)	0.43 cm s <sup>-1</sup>	_	0.43 cm s <sup>-1</sup>

<sup>\*</sup> S air concentration measured at Framlingham.

<sup>†</sup> S air concentration not measured at Rothamsted.

thousandth acre drain gauge during 1969, the hundredth year. The SO<sub>4</sub> sulphur collected in drainage through this gauge was 58.36 kg S ha<sup>-1</sup>. Subtracting sulphur content of rainwater (33.02 kg S ha-1) we obtain 25.34 kg S ha-1 which represents dry deposition plus a small amount of sulphur mineralised from soil organic matter. Ignoring mineralised sulphur, we have calculated a mean sulphur deposition rate of 8.03 × 10<sup>-12</sup> g cm<sup>-2</sup> s<sup>-1</sup> yr<sup>-1</sup> which, allowing for differences in air SO<sub>2</sub> concentrations between 1969 and 1974, agrees well with the figure obtained here  $(8.72 \times 10^{-12} \text{ g cm}^{-2} \text{ s}^{-1} \text{ S})$ .

Deposition velocities of sulphur with respect to vegetation have been shown to be two or three times faster4,5 so that, the amount of sulphur deposited on vegetation would be correspondingly greater than the amounts measured here.

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### Fluorocarbon anaesthetics break hydrogen bonds

Many fluorocarbon derivatives that contain higher halogens dissociate hydrogen bonds of the N-H-N, O-H-O, S-H-S, N-H-O=C type which are similar to those occurring in living organisms. A parallelism exists between this property of fluorocarbons and their anaesthetic potency. Their interference with cell division might also be related to it.

Halothane (CF<sub>3</sub>-CHClBr) is the most widely used inhalation anaesthetic1-3. The mechanism of the anaesthetic action of these compounds has not yet been elucidated, although anaesthetic potency has been related to lipid solubility<sup>4,5</sup>, clathratehydrate formation<sup>6,7</sup>, and interaction with proteins to produce conformational changes<sup>8-10</sup>. We have observed that fluorocarbons can 'break' hydrogen bonds11,12 and have investigated the relationships between this property and their anaesthetic properties by measuring infrared spectra of systems which are hydrogen bonded in solvents of chlorine, bromine or iodine containing fluorocarbons. These systems were hydrogenbonded amides (N-ethylacetamide, N-methylbenzamide, acetanilide, δ-valerolactam), alcohols, amines and thiols. An order of potency of hydrogen-bond breaking has been established from the infrared band intensities: F < Cl < Br < I. This applies to all N-H-N, O-H-O, S-H-S and N-H-O=C type hydrogen bonds. It is very significant, in our opinion, that F < Cl < Br < I is also the order of increasing anaesthetic potency1,3.

Another observation deserves consideration. If the fluorocarbon contains a hydrogen, its potency of hydrogen-bond breaking greatly increases. Although CF<sub>3</sub>-CCl<sub>3</sub> has no significant ability to break hydrogen-bonds CF3-CHCl2 is about as potent as halothane (see Fig. 1).

In view of these two parallels between hydrogen-bond breaking and anaesthetic potencies it is natural to speculate that the breaking of hydrogen bonds is an important step in the mechanism of anaesthesia which has been induced by fluorocarbons. The hydrogen bonds that may be 'broken' can be water-water or water-protein bonds or hydrogen bonds within

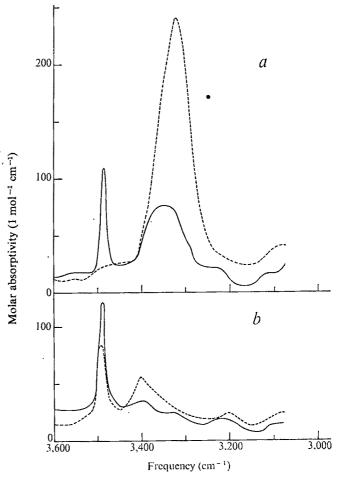


Fig. 1 The infrared spectra of a 0.045 M solution of N-methylpivalamide in a 1:1 mixture of CCl<sub>3</sub>F and methylcyclohexane (dashed line) and in the same solvent with 1.02 M CF<sub>3</sub>-CHCl<sub>2</sub> added (solid line). a, At -80° C; b, at 23° C.

the protein and nucleic acid structures; they all have energies similar to those of our model systems. In some cases the hydrogen bonds may actually be dissociated, in others a lesser perturbation may be sufficient.

The importance of this observation may well go beyond the mechanism of anaesthesia. Nunn et al.13, and Jackson14 have reported that halothane arrests mitosis. Mitosis goes through several phases involving both the breaking and the formation of the hydrogen bonds which keep the two parts of the nucleic acid double helix together. In view of the potent ability of fluorocarbon anaesthetics to break hydrogen bonds it is tempting to speculate that the former interfere with cell division by perturbing or breaking hydrogen bonds around or in the helices, or in the mitotic spindle.

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# Radiocarbon chronology for Seibal, Guatemala

The significance of the study paper reported here is the fact that the origins of Maya civilisation are pushed much further back into the past. Radiocarbon dating of the earliest and latest states of the Mayan 'city' centre of Seibal indicate an origin as early as 900 BC, declining by about 1000 AD. This discovery narrows the interval between the beginning of lowland Maya occupation and the Olmec centres on the Gulf of Mexico, allowing for the coexistence of quite different cultures in relatively close geographical proximity.

Seibal, located on a bluff in the upper Usumacinta-Pasión river drainage of Guatemala (approximately 16°N, 90°W), is one of the important lowland Maya 'city' centres excavated in recent years by Harvard's Peabody Museum<sup>1,2</sup>. Radiocarbon dating of charcoal and bone samples at the University of California (UCLA) indicate that the city has been in existence for almost 2,000 yr, roughly symmetric about the AD-BC division3. Seibal's time was over by 10.5.0.0.0 katun, equivalent to 928 AD, even though some population lingered on for some time. A more detailed chronology is based upon the following radiocarbon measurements in conjunction, where applicable, with Mayan dates, which in turn are related to our present calendar via the Goodman-Martinez-Thompson correlation (Fig. 1). All radiocarbon samples were pretreated to remove impurities and analysed, where appropriate, by their collagen content, precautions being taken for isotopic purity<sup>4</sup>. A comparison showing the concordance of charcoal- and collagenbased radiocarbon dates has appeared elsewhere<sup>5</sup>.

The earliest occupation discovered belongs to the Real Xe phase of the Middle Preclassic Period. In particular, a sample of charcoal was dated associated with pottery vessel 2 in cache 7 of Central Plaza group A. This cache was actually located beneath several preclassic floors and definitely sealed in a Real Xe phase context. It consisted of a cruciform arrangement of pottery and jades. Among the latter was a blood letter resembling an ice pick in shape, a style associated with the Olmec horizon and Olmec sites of the Gulf Coast. These Olmec sites date from as early as about 1300 BC and persist as late as the fifth century BC. For the purposes of this paper the dates have been tree-ring calibrated, based on an earlier publication which did not include this calibration.

Previous archaeological estimates of the date of the Xe ceramic sphere derived from radiocarbon dates at Altar de Sacrificios<sup>7</sup> and other lines of evidence, were in the range 900-600 BC. The present charcoal date of 2,610 ±75 yr (UCLA: 1437; S-382b) can be calibrated to correspond to an age of 900 BC; this, in a general way, is in line with estimates for the early Middle Preclassic Period of Mesoamerica as a whole<sup>8,9</sup>. In relation to chronology, Real Xe and the Xe ceramic sphere<sup>10</sup> immediately precede the Mamom ceramic sphere.

After the Real Xe phase, the next date obtained is based on a bone sample from burial 19 found beneath a platform in front of structure D-3. Associated pottery places this burial in the Tepejilote phase of the Tepeu ceramic sphere. The particular pottery types indicate a Tepeu 2 placement, or, at the earliest, a late Tepeu 1. The radiocarbon'date obtained from collagen is 1,490 ± 320 yr (UCLA: 1640D; S-1316) corresponding to a calibrated age of 500 Ad. Because of the small sample size the statistical error is larger than desirable, which explains why this particular date does not agree with the archaeological estimate of about 675-830 Ad. This is based on the 11.16.0.0.0 correlation and ceramic styles<sup>11</sup>, with a preference for the earlier end of this range.

The next datum is a bone sample obtained from burial 17 located in structure A-2. The structural context indicated an Early Classic dating, although the pottery with the burial pertained to the Tepejilote phase and could very well have been the earliest part of that phase, equivalent to a Tepeu 1 date. The radiocarbon date of 1,420 ± 95 yr (UCLA: 1640C; S-1193) corresponded to a calibrated age of 600 AD, in agreement with the inception of Tepeu 1 in the 11.16.0.0.0 correlation.

Burnt human bone from a mass burial (No. 4) in structure A-13 provided the next date. The radiocarbon date is 1,100  $\pm$  100 (UCLA: 1,640F; S-410) which can be calibrated to 930 AD. Structure A-13 is an interesting small mound or platform that seems to have been designed to receive a mass interment. It is located immediately next to a large ball-court in the Central Plaza of group A. Perhaps the structure does indeed contain the sacrificed remains of persons who had been in some way involved with the game and actually may have been the defeated team. On the basis of associated potsherds, the mass burial can be dated to the Bayal phase of the site's history. Bayal, which can be placed at about 10.0.0.0.0 to 10.5.0.0.0 (830-928 AD), was a time of considerable ceremonial activity at Seibal including architectural construction and dedication of stelae, with monuments dated as late as 10.3.0.0.0 (889 AD). Characteristic of the Bayal phase is the fine Orange pottery of group Y or Altar series.

Similarly, a bone sample from burial 12, which was found below the patio floor of structure 31A, again confirmed the Bayal phase. The building is a small one on the outer periphery of Seibal, as opposed to the main ceremonial centre. The radiocarbon date is  $1,000 \pm 65$ , calibrated at 1000 AD (UCLA: 1640A), all the associated sherds belong to the Bayal phase, making the radiocabon dating late for the Bayal period of 830-928 AD, although it is quite possible that some of the outlying structures at Seibal were occupied for a few decades beyond the estimated date of 10.5.0.0.0 katun (928 AD). Activities in the main ceremonial centre were over by 10.4.0.0.0 or 10.5.0.0.0 but secular life may have continued in the outskirts.

Additional confirmation for the age of the Bayal phase is derived from a bone sample from burial 13, found inside a small structure (26d) also on the periphery. An associated burial and its surrounding sherd also indicate the Bayal phase.

Table 1 Correlation between ceramic spheres, phases and tree-ring calibrated radiocarbon dates at Seibal

Ceramic sphere	Phase	Calibrated radiocarbon date (12)
Boca	Bayal	1050 AD 1000 930
Tepeu	Tepejilote	600 (500) AD
Tzakol Chicanel Mamom		
Xe	Real Xe	900 вс

Early start of Real Xe at Seibal narrows the interval between the beginning of lowland Maya occupation and Olmec sites on the Gulf of Mexico.

The radiocarbon date is 940+70 (UCLA: 1640B) calibrated to 1050 AD, in agreement with previous considerations.

On balance, the calibrated radiocarbon dates agree well with lowland Maya Middle Preclassic and Terminal Late Classic estimates. The latter are consistent with an 11.16.0.0.0 correlation of Maya and Christian calendars. Two dates, one from the earlier late Classic Period, the other from what would appear to be the equivalents of a Tepeu 1 or 2 context, are a little more difficult to reconcile with the 11.16.0.0.0 correlation, suggesting, instead a 12.9.0.0.0 correlation. The latter of these two dates does harmonise with Tepeu 1, as dated in the 11.16.0.0.0 correlation, however, while the statistical error of the former still allows for the same interpretation.

Unfortunately, the Early Classic Period (Tzakol ceramic sphere) is very poorly represented at Seibal and no samples from such a context could be obtained. The Late Preclassic (Chicanel ceramic sphere) is, on the other hand, well represented by structures and pottery at the site although we were unable to find appropriate materials for radiocarbon dating.

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# Fever in the lizard Dipsosaurus dorsalis

Fever is considered to be a universal response of warm-blooded animals to endotoxins1. Although during a fever a mammal uses behavioural as well as physiological means to increase its body temperature<sup>2</sup>, it is not known whether fever develops in an animal such as a lizard which regulates its body temperature largely by behaviour. For example, the desert iguana (Dipsosaurus dorsalis) regulates its body temperature close to 38.5° C if placed in a chamber with a temperature gradient3. If this lizard is placed in a temperature chamber in which one end is heated to above the animal's lethal body temperature (50° C) and the other end is maintained at room temperature, the lizard regulates its temperature by moving back and forth between the two sides4. Under these conditions, one can determine its high and low set-points (Fig. 1). The central nervous control of temperature in an ectotherm, such as a lizard, and an endotherm, such as the rabbit, appears to be quite similar. For example, both possess a hypothalamus which is thermally sensitive<sup>5,6</sup>, and lesions in the posterior hypothalamus in both lizards4 and mammals<sup>7</sup> lead to an inability to maintain a high body temperature.

Because of the similarities in the central nervous control of thermoregulation in reptiles and mammals, and because fever in mammals is accompanied by major behavioural adjustments, we suspected that fever could be produced in a reptile. We now report that bacteria that produce fever in a rabbit will produce a similar fever in the lizard Dipsosaurus dorsalis.

Lizards weighing 25-60 g (Hermosa Reptile Farm, Hermosa, California) were housed in circular cages and fed meal worms, lettuce and water ad libitum. The cages and experimental chambers were kept on a 12-h light and 12-h dark photoperiod. The chamber was heated by a 250 W heat lamp which was also on a 12:12 cycle.

Experiments were carried out in a temperature-controlled room. Each lizard was placed in a wooden box (30 cm ×  $140 \, \mathrm{cm} \times 30 \, \mathrm{cm}$ ) of which one end was at the room temperature of 30° C and the other end at 50° C. This high temperature was provided by heating coils taped to the undersurface of the floor of one end of the box. The two sides of the box were separated by a small wire mesh bridge to provide a clear boundary between the two temperature extremes. A copper-constantan thermocouple covered with polyethylene tubing (PE 100) was placed about 3 cm into each lizard's cloaca and taped to its tail; this did not noticeably impair the movement of the lizards. Thermocouples were connected to a Honeywell Electronik 112 multipoint recorder which recorded the temperature of each lizard ( $\pm 0.1^{\circ}$  C) every 30 s.

Aeromonas hydrophila, Gram-negative bacteria pathogenic to reptiles and amphibians8, were grown on blood agar and killed by washing in 70% ethyl alcohol. They were then centrifuged and resuspended in physiological saline. The concentration of bacteria was determined by a turbidity test.

Lizards were allowed 1 d to adapt to the wooden box, and on the second day control data were recorded. On the third day either 0.2 ml of a solution containing 2 × 1010 bacteria per ml of saline or 0.2 ml of sterile physiological saline alone was injected into the heart using a 1.5-inch 26-gauge needle. Blood

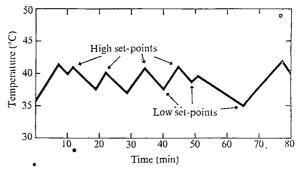


Fig. 1 Record of the cloacal temperature of Dipsosaurus dorsalis regulating its temperature in the wooden box in which the substrate at one end was maintained at 30° C and the other end at 50° C. At the high set-point, the lizard moved from the warm side of the chamber to the cool side. At the low set-point the lizard moved back to the warm side of the chamber. Data were recorded every 30 s. The high and low set-points, for any time period, represent the average of these points over that time period.

was redrawn into the syringe to ensure that the needle was in the heart. All injections were done at the same time of day to avoid possible effects of diurnal variations.

To determine whether increases in body temperature were due entirely to behavioural modifications, or whether an increased internal production of heat was part of the response, three lizards were given injections of bacteria identical to the above. They were then placed in a wooden chamber (14 cm  $\times$  30 cm  $\times$ 30 cm) which was held at 30° C. Cloacal temperatures were measured as before.

Four New Zealand white rabbits (Oryctolagus cuniculus) were used to determine whether A. hydrophila could also produce fever in mammals. The rabbits' tails were shaved and a thermocouple was inserted 10 cm into the rectum and taped to the tail. The rabbits were placed in a restrainer and allowed 1 h to acclimatise to room temperature (22° C). Then 0.2 ml of saline was injected into the marginal ear vein. After 1 h (control period), 1 ml of 3 × 10<sup>8</sup> bacteria per ml saline was injected into the marginal vein of the other ear.

In 10 lizards, given free choice of either the 50° C or 30° C environment, injection of 4 × 109 bacteria produced little increase in temperature during the first few hours. Body temperature increased approximately 2° C between the fourth and sixth hours (Fig. 2). The increases of the average high (41.1° C to 42.7° C) and low (37.4° C to 39.7° C) set-points were highly statistically significant (P<0.001 using paired sample analysis).

When the same concentration of bacteria was injected into three lizards maintained in a constant temperature chamber at 30° C, their temperatures did not change throughout the control and experimental periods. Injection of saline into nine lizards, given free choice of the warm or cool environment, produced no changes in high or low set-points (Fig. 2). Comparison of lizards injected with saline to those with A. hydrophila revealed a statistically significant increase in the high (P < 0.02)and low (P < 0.003) set-points during the second 3 h (Student's t test). Injection of saline into the four rabbits produced no increase in rectal temperature. Injection of the bacteria produced a fever with a latency of about 20 min and a mean maximum rise of 2.2° C within 3 h.

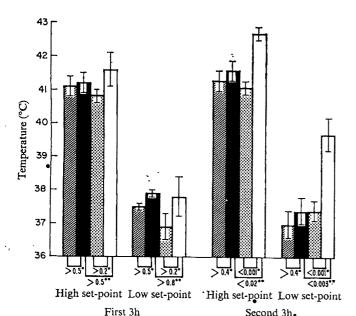


Fig. 2 Average high and low set-points ( $\pm$ s.e.m.) during control periods (day 2) and after intracardiac injection of 0.2 ml isotonic saline or 0.2 ml of 2  $\times$  10<sup>10</sup> Aeromonas hydrophila ml1- (day 3) into Dipsosaurus dorsalis. \*, Level of significance using paired sample analysis; †, level of significance using Student's t test. Hatched columns, control period before saline injections; solid columns, period after saline injections; stippled columns, control period before Aeromonas injections; open columns, period after Aeromonas injections.

These data indicate that a bacterium that causes fever in a rabbit has a similar effect in a lizard. Since the lizards could not increase their temperature in response to the bacteria when the ambient temperature was held constant, fever in these lizards cannot be developed by increased internal production of heat. The relatively long latency before the onset of fever in the lizards might be due to the lower metabolic rate of an ectotherm in comparison with that of an endotherm.

These results demonstrate that fever can be sustained by behavioural regulation in an ectotherm. Also, since the hypothalamic control over thermal responses in reptiles and mammals are similar4-6 and as we have shown, a similar concentration of bacteria will produce a similar increase in temperature in both a reptile and a mammal, we suspect a common origin for reptilian and mammalian fever. If this is true, then the ability to develop a fever existed before the time when evolutionary lines for mammals and reptiles diverged. This suggests that at least the behavioural component of fever had evolved by the late Palaeozoic or early Mesozoic, or perhaps even earlier. We cannot rule out the possibility, however, that fever might have independently and perhaps recently evolved in reptiles and mammals.

These findings also open new possibilities for ascertaining the adaptive value of fever. The question of whether fever is beneficial or harmful to the host has been difficult to resolve in mammals. Since the increase in body temperature in response to bacterial infection might have evolved in primitive reptiles, or even in amphibians, the adaptive value of fever might be revealed by a careful study of the role of the febrile response in these classes of vertebrates.

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### Dependence of juvenile hormone release from corpus allatum on intraglandular content

THE corpus allatum has been shown to be the source of chemically defined juvenile hormones in five different species of insect1-5. The release of juvenile hormone from active corpora allata is known to be a prerequisite for the rapid induction and promotion of vitellogenesis in adult female insects of several orders, including all tested members of the Orthoptera<sup>6,7</sup>. It is generally believed that temporal patterns of activity in the adult female corpus allatum are of prime importance in initiating and maintaining oocyte growth<sup>6,8</sup>.

Up to now the activity of the corpus allatum has been inferred from indirect observations such as glandular activity

after transplantation into a suitable host animal<sup>9</sup>, gland extirpation plus hormone replacement therapy<sup>10</sup> and measurement by bioassay of apparent titres of juvenile hormone in the haemolymph<sup>11,12</sup> and in freshly excised glands<sup>12</sup>. A direct, quantitative method for estimating the activity of the corpus allatum has recently become available, following the demonstration that the synthesis and release of juvenile hormone from corpora allata taken from adult female \$\mathcal{S}\$chistocerca gregaria can be rapidly measured in vitro by precise radiochemical procedures<sup>4,13</sup>. Here we deal mainly with the possible regulation of release of newly synthesised juvenile hormone using corpora allata from female \$S\$. gregaria during the first gonotrophic cycle. We show that at all times during sexual maturation it is the rate of synthesis of juvenile hormone and not the rate of release, which determines the endocrine activity of the glands.

To explore the relationship between the rate of synthesis of locust juvenile hormone, methyl 10,11-epoxy-3,7,11-tri-methyl-2,6-trans,trans-dodecadienoate (C<sub>16</sub>JH), the extent of its storage within the glands and its rate of release, freshly-isolated corpora allata were incubated in TC 199 medium containing methyl-14C-methionine for 2 h at 30°C with shaking. The medium was separated from the washed glands and both were analysed for radiolabelled C16JH content as previously described13. Observations were made on glands taken daily from adult female locusts between the ages of 3 and 12 d after fledging, during which period there are changes of approximately 400-fold in the rate of juvenile hormone release measured in vitro (S. S. T., and G. E. P., unpublished). We also carried out parallel measurements on glands incubated with optimal concentrations of C-23H-trans, trans farnesenic acid, an exogenous precursor known to stimulate directly the rate of juvenile hormone biosynthesis in these glands4. Thus, on each day of sexual maturation, we compared the steady state glandular content of C16JH with its rate of release from the glands, both in glands synthesising juvenile hormone spontaneously and in those whose rate of synthesis has been stimulated artificially with exogenous farnesenic acid. In this way we tested by direct observation, the alternative hypotheses that the naturally variable rates of release of hormone from the glands are the result either of direct regulation of the rate of biosynthesis, or of the direct regulation of the rate of release of newly synthesised hormone. Figure 1 shows the rates of release of hormone as a function of intraglandular content. For each day the points representing spontaneous activity and farnesenic acid-stimulated activity have been joined with a straight line, whose slope we term the 'release coefficient'. These slopes indicate the extent to which stimulating JH biosynthesis, by addition of farnesenic acid to the medium, alters the relationship between glandular content and rate of release. It can be seen that the data approximate a linear relationship of 'rate of release' on 'glandular content'; analysis shows that the regression line passes through the origin and has a slope of 2.7 h<sup>-1</sup>. Moreover, this mean regression is reflected in the 'release coefficients' of the various glands having these widely different spontaneous activities (mean = 2.83 h<sup>-1</sup>; s.d.  $\pm 1.00$ ). Although the variations in slope are small compared with the variations in spontaneous activity of the glands, the possibility existed that the observed variations in 'release coefficient' might have some functional relationship with the spontaneous in vitro activity of the glands. We have therefore compared these two parameters (Table 1) and regression analysis indicated a very weak correlation (slope = 0.046; s.d.  $\pm 0.023$ ) indicating that differences in 'release coefficient' are attributable to independent variations including experimental error.

Thus at all times during sexual maturation, both the rates of synthesis and release of juvenile hormone can be stimulated proportionately by the addition of farnesenic acid to the medium, such that the hourly release of newly-synthesised juvenile hormone is approximately equal to 2.7 times the steady state glandular content of the hormone. This seemingly simple relationship between the rate of release of juvenile

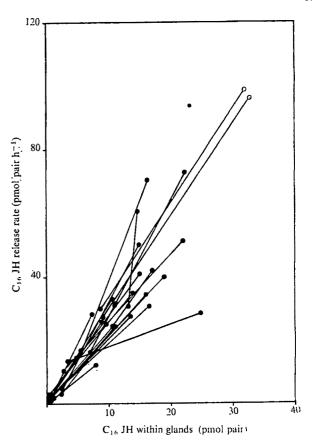


Fig. 1 The rate of release of C<sub>16</sub>JH relative to its concentration within the corpora allata. Each point represents the mean of two sets of observations on single pairs of glands from adult female locusts during the first gonotrophic cycle. The lines join observations from glands synthesising  $C_{1a}JH$  spontaneously and those stimulated with  $^3H$ -farnesenic acid on each given experimental day. In every case the rate of release from stimulated glands is higher than the spontaneous rate. Both the rate of release and the glandular content of hormone were monitored throughout the period of sexual maturation by incubation of glands for 2 h in TC 199 (20 mM HEPES) containing Ficoll (20 mg ml<sup>-1</sup>) and either <sup>14</sup>C-methionine (final specific radioactivity 36.7 mCi mmol<sup>-1</sup>) or <sup>14</sup>C-methionine plus <sup>3</sup>H-farnesenic acid (20 µM; specific radioactivity 25 mCi mmol<sup>-1</sup>). After incubation, individual pairs of glands were separated from the medium and both glands and medium were separately extracted with fifteen volumes of CHCl<sub>3</sub>:C<sub>2</sub>H<sub>6</sub>OH (5:1) after adjustment to pH 4.6 and addition of appropriate nonradioactive marker compounds. Extracts were analysed by thin layer chromatography (Merck F<sub>254</sub> silica gel) in ethyl acetate: benzene (25:75) followed by episcopic fluorescence quenching densitometry and scanning gas-flow radiometry. Radioactivity in  $C_{18}JH$  was quantified by liquid scintillation spectrometry. At all times the rate of release of juvenile hormone is determined by the glandular content of hormone. ( $\alpha = 0.0$  pmol h<sup>-1</sup> per pair of glands;  $\beta = 2.7$  h<sup>-1</sup>; r = 0.92).

hormone and the steady state glandular content, irrespective of the spontaneous activity of the glands, suggests that the export of juvenile hormone is governed by laws of simple physical diffusion. Although the route of export of juvenile hormone is not yet established, it has been proposed that this may involve diffusion out of the glands through a network of extracellular spaces.

Ultrastructural observations on the corpora allata of this <sup>14</sup> and other <sup>9,15</sup> insect species have led others to suggest an association between the release of juvenile hormone and observable changes in the subcellular architecture of the glands. It has been suggested that in the corpus allatum of Calliphora erythrocephala newly synthesised juvenile hormone accumulates initially in vacuoles associated with the agranular reticulum. These vacuoles eventually break down into lipid droplets

Table 1 Relation between rate of release of C<sub>16</sub>JH synthesised spontaneously and its 'release coefficient'

Rate of release of C <sub>16</sub> JH	Release coefficient	-
synthesised spontaneously	$R_{\mathrm{st}} - R_{\mathrm{sp}}$	Adult age
(pmol h <sup>-1</sup> per pair of glands)	$\overline{C_{\mathrm{st}}-C_{\mathrm{sp}}}^{*}$	(d)
29.90	3.28	9
16.70	5.11	12
14.63	2.92	8
12.09	3.80	10
10.40	2.11	8
4.70	2.16	9
4.40	3.68	10
3.08	2.34	7
2.80	2.64	7
2.03	1.95	6
1.83	1.75	12
1.80	2.07	6
1.80	3.88	4
0.80	2.85	5
0.44	2.86	. 6
0.43	2.30	5
0.14	2.01	5
0.11	2.93	4
0.07	3.15	6 4 5 5 5 5 4 4 3
0.05	2.88	3

Each value represents the mean of two separate sets of observations on single pairs of glands from animals of the age indicated. There is no correlation between spontaneous rate of hormone release and 'release coefficient' ( $\alpha = 2.59$ , s.d.  $\pm 0.21$ ;  $\beta = 0.046$ , s.d.  $\pm 0.023$ ;

\* $R_{\rm st} = C_{16}$ JH release rate of glands stimulated by addition of sh-farnesenic acid;  $R_{\rm sp} = C_{16}$ JH release rate of glands synthesising hormone spontaneously;  $C_{\rm st} = C_{16}$ JH content of glands stimulated by addition of sh-farnesenic acid;  $C_{\rm sp} = C_{16}$ JH content of glands synthesising hormone spontaneously.

within the cytoplasm and are then released into the haemolymph<sup>15</sup>. On the other hand, in Locusta migratoria, the fragmentation of the smooth endoplasmic reticulum is believed to be an important stage in the release of hormone from the glands9. It has also been reported that corpora allata of S. gregaria contain lipid droplets which increase in size towards the periphery of the cells<sup>14</sup>. It is difficult to reconcile the results presented here with any proposed mechanism of hormone release involving massive changes in the ultrastructural organisation of the cells, particularly since it is known that in S. gregaria newly synthesised juvenile hormone can be detected in the culture medium within 10 min of the addition of radiolabelled precursors<sup>13</sup>. Therefore we propose that the observed changes in the ultrastructure of cells of the corpus allatum reflect changes in their hormone synthetic capacity, rather than reveal the morphological basis of hormone synthesis, intracellular transport and release.

The possibility that storage of newly synthesised juvenile hormone may be an important facet of the normal endocrine function of the corpus allatum has never been directly tested in any species of insect. It is now clear that at no time during the course of rapid sexual maturation in the adult female desert locust do isolated corpora allata synthesise juvenile hormone and then fail to release it into the medium. Whether or not this also holds true in circumstances other than those of rapid sexual maturation remains to be seen.

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#### Choline transport by neuroblastoma cells in tissue culture

NEUROBLASTOMA cells in tissue culture have been used widely as an in vitro model for neural functions. Certain 'cholinergic' clones have raised choline acetyltransferase activities1, and all clones studied display a high affinity uptake system for exogenous choline<sup>2</sup>. Whereas low affinity  $(K_T \simeq 10^{-3} \text{ M})$  choline transport systems occur in various neural and non-neural mammalian tissues, high affinity ( $K_T < 10^{-5}$  M), sodiumdependent choline uptake in brain synaptosomes3,4 and the guinea pig intestine (C. B. Pert, and S. H. Snyder, personal communication) is restricted to cholinergic neurones. Efficient conversion of choline to acetylcholine, ionic requirements and sensitivity to inhibitors should uniquely distinguish the cholinergic transport system from those of similar affinity for choline which occur in many cultured mammalian cell lines. We have examined these properties of the choline transport system in cholinergic and non-cholinergic neuroblastoma clones.

The profiles for enzymes involved in the synthesis and degradation of acetylcholine are characteristic of each cloned neuroblastoma cell line (Table 1). The activities of choline acetyltransferase which we obtained are similar to other values previously reported for these lines<sup>1</sup>, although the N18 clone is

Table 1 Acetylcholinesterase and choline acetyltransferase activities of neuroblastoma cell lines

Cell line	Acetylcholinesterase	Choline acetyltransferase
	nmol per mg protein per min	nmol per mg protein per h
NS20 clone		6.50
N18 clone		3.71
N1E clone	TF. 7	n.d.
MIE CIOILE	7.	111-04-

n.d., Not detectable.

Neuroblastoma cells were obtained from Microbiological Associates (clone N18) or from Dr Arthur Blume (clones N1E and NS20). The nomenclature used for the clones is that of Amano et al.1. The lines were maintained by serial cultivation in Dulbecco's modification of Eagle's medium supplemented with 10% foetal calf serum. For enzyme assays and metabolic studies, the cultures were allowed to reach stationary phase, giving about  $2 \times 10^7$  cells per 75 cm<sup>2</sup> plastic flask (Falcon Plastics, Inc.). Acetylcholinesterase was measured by a contract of the property of modification of the method of Ellman et al.11 in which we use 1% Nonidet P-40 to solubilise the enzyme and assay activity at 35° C Choline acetyltransferase activity was determined by a standard technique12.

Table 2 Kinetic constants for choline transport by neuroblastoma cells

1	Cell line NS20 N18			NIE		
Constant $K_T$ (M × 10°) $V_{\text{max}}$ (M × 101° per min per mg protein)	Unstarved 20.7 19.8	Starved 4.5 21.2	Unstarved 17.1 38.9	Starved 3.5 11.2	Unstarved 10.2 2.8	Starved 2.1 3.0

Neuroblastoma cells of the clones indicated were grown to saturation in Linbro microwells (about 0.4 mg of protein or  $8 \times 10^5$  cells per well). Triplicate cultures were either incubated overnight in Hank's BSS to deplete the cells of choline and labile choline metabolites or they were merely washed twice with the buffer. For measurement of choline transport, cultures were exposed to 0.2 ml of Hank's BSS containing the appropriate concentrations of <sup>3</sup>H-choline (from Amersham Searle at a specific activity of 0.5 Ci mmol<sup>-1</sup>) for 4 min at 37° C and then quickly washed three times with ice-cold buffer. Cells were lysed by addition of 0.5 ml of distilled water, and radioactivity in the suspension was determined by scintillation counting in dioxane based phosphor. Choline accumulation was linear for nearly an hour in all three clones using both choline depleted cells and those maintained in medium containing choline. After a 1-h exposure to  $5 \times 10^{-5}$  M <sup>3</sup>H-choline, the concentration of labelled compounds within cells exceeded the radioactivity in the medium by a factor of 20. The kinetics of accumulation and cell/medium ratios were very similar to those reported for lymphoblasts *in vitro*<sup>7</sup>. It was estimated that at the highest choline concentration used, passive diffusion of choline into cells accounted for approximately 5-10% of the total uptake. After correction for diffusion, the data were plotted and linear regression lines calculated. The difference between  $K_{\rm m}$  values obtained with and without choline depletion is highly significant (P < 0.001) while the difference between the  $V_{\rm max}$  values is not (0.3 > P < 0.5)

apparently not 'inactive' in respect of synthesis of this enzyme. We consistently found higher cholinesterase activities in induced cultures of our clone N18 and lower activities in our clone N1E than are usually reported<sup>1,5</sup>, especially when cells of the N18 line were induced for acetylcholinesterase by leaving serum out of the growth medium<sup>6</sup>. These differences may be attributed to variations in the assay procedure or growth conditions and would not be expected to influence the results of the present study.

 $K_{\rm T}$  values for choline transport were determined in three clonal lines with and without previous choline depletion (Table 2). The affinities of the transport systems for choline in all three clones are quite similar and are increased about fivefold by choline depletion. The  $K_{\rm T}$  values for starved and unstarved cells are similar to those reported for hepatoma cells by Plagemann<sup>8,9</sup> and for starved neuroblastoma cells<sup>2</sup>. Experiments using choline concentrations as high as  $2\times 10^{-3}$  M did not reveal a  $K_{\rm T}$  of lower affinity in depleted cells so it must be assumed that there is only one transport system for choline in neuroblastoma cells and that its affinity depends upon previous exposure of the cells to choline.

 $V_{\rm max}$  values for the adrenergic clone (N1E) indicate a lower capacity of its transport system, a reflection, perhaps, of smaller surface area as a result of relatively poor axonation by this clone. In general, the data are consistent with those obtained using hepatoma cells. The  $V_{\rm max}$  values with and without choline depletion are similar to those previously observed in neuroblastoma cells. Double reciprocal analysis of rates of uptake by each of the 3 clones indicated competitive kinetics. This apparent competitive inhibition cannot be due to isotope dilution by leakage of non-radioactive free choline from unstarved cells since there is not sufficient intracellular free choline to produce the observed effect.

We then studied the fate of choline transported into the various clones (Table 3). Only very small amounts of choline were detected in any of the clones. This is in marked contrast to brain cholinergic synaptosomes which accumulate choline and acetylcholine in roughly equal proportions<sup>3,4</sup> when exposed to a similar concentration of free choline. The formation of small quantities of acetylcholine was, however, significantly dependent on the activity of intracellular choline acetyltransferase (Table 1), being low in the adrenergic clone (N1E), intermediate in the 'inactive' clone (N18) and highest in the cholinergic clone (NS20). The major radioactive product in all 3 clones was phosphorylcholine which, as suggested by data obtained with the long incubation period, was capable of serving as a precursor of phosphatidyl choline. Subsequently, more detailed experiments showed that the free choline could be chased first into phosphorylcholine and then into phosphatidyl choline.

Disposition of choline in this manner would be expected of

cells lacking either the 'high affinity' choline transport system itself or proper coupling to choline acetyltransferase. To distinguish between these possibilities, we examined the effects of various known inhibitors of transport on uptake and metabolism of choline. Synaptosomal uptake of choline at low concentrations is sensitive to hemicholinium<sup>3</sup> and dependent on sodium<sup>4</sup>. As Table 4 shows, the neuroblastoma transport system is not inhibited by even a high concentration of hemicholinium and is independent of sodium. The distribution of choline among its various metabolites is also unaffected by these treatments. It seems that although cultured neuroblastoma cells have a transport system for choline, it is similar to that found in many types of non-neural cells and does not display the characteristics associated with the 'high affinity' uptake system unique to brain cholinergic synaptosomes.

Table 3 Fate of choline in neuroblastoma cell lines

	4-min Incorporation			12-h Incorporation		
	N18	NS20	NIE	N18	NS20	NIE
Choline	3.6	2.8	4.1	0.5	1.0	1.3
Acetylcholine	0.2	0.5	0.1	0.1	0.4	0.1
Phosphorylcholine	96.2	96.7	95.9	72.2	71.0	85.2
Phosphatidyl choline	0.1	0.1	0.1	27.2	27.7	13.5
Total	100.1	100.1	100.2	100.0	100.1	100.1

Neuroblastoma cells were grown to confluence in 75 cm<sup>2</sup> culture flasks (Falcon Plastics, Inc.) and depleted of choline by incubation overnight in Hank's BSS containing an irreversible inhibitor of AChE<sup>6</sup>.  ${}^{3}\text{H-choline}$  was added at a final concentration of 5  $\times$  10<sup>-5</sup> M and incubation continued for the indicated times. Monolayers were washed three times with ice-cold buffer and then scraped off into 0.5 ml of ice-cold 95% ethanol containing 0.2% acetic acid, 2 mM acetylcholine, 3 mM choline and 0.1 mM neostigmine<sup>3</sup>. The suspension was homogenised thoroughly and the debris pelleted at 1,000g for 10 min. The pellet was re-extracted with acid ethanol and then with ethanol-ether (1:1). The ethanol-ether extract was shown chromatographically to contain mostly phosphatidyl choline and a very small amount of phosphoryl choline<sup>13</sup>. Phosphoryl choline, acetylcholine and free choline were all soluble in the acid-ethanol extract but could be separated by high voltage paper electrophoresis14. Since phosphoryl choline remained at the origin in our electrophoretic system, its identity was confirmed by thin layer chromatography". In 4 min, an average of 4,000 c.p.m. per mg protein were incorporated, increasing to approximately 10,000 c.p.m. per mg protein after 12 h. Uptake by the various lines was quantitatively similar so, to facilitate comparisons, data are expressed as % total c.p.m. recovered. Assuming that complete equilibration of the acetylcholine and phosphorylcholine pools with exogenous labelled choline occurred during 12-h incubation, then the intracellular acetylcholine concentration in NS20 can be calculated to be about 10-7 M and that of phosphorylcholine about  $2\times10^{-6}$  M. Under the conditions of these incubations, greater than 99% of acetylcholinesterase activity was inhibited. In the absence of an acetylcholinesterase inhibitor even less acetylcholine was recovered, but an accurate determination of the acetylcholine concentration under these conditions could not be made.

Table 4 Effect of various inhibitors on choline transport

Inhibitor	Choline uptake
	(% of control)
None	100
Hemicholinium (0.1 mM)	131
Na <sup>+</sup> replaced by Li <sup>+</sup>	81
Na <sup>+</sup> replaced by sucrose (0.25 M)	66
Ca <sup>2+</sup> replaced by Mg <sup>2+</sup>	93
2,4-dinitrophenol (1 mM)	40
Ouabain (Î mM)	38
Incubation at 0° C	10

Neuroblastoma cells (NS20 clone) were grown to confluence in 75 cm² plastic flasks and depleted of choline metabolites as described for Table 3. The cultures were preincubated with the anticholinesterase agent and the indicated metabolic inhibitor in Hank's BSS for 15 min. Then  $2 \times 10^{-3} M$  <sup>3</sup>H-choline was added and after a further 15 min incubation the cells were collected as described in Table 2.

As would be expected for any energy-dependent cellular process, uptake is inhibited by low temperature and 2,4dinitrophenol. But this should not be interpreted as evidence. that choline is taken into neuroblastoma cells by active transport. We have never observed an intracellular choline concentration greater than that in the medium and inhibition of transport by such nonspecific agents could have other explanations. In erythrocytes there is a facilitated diffusion system for choline10 and we have no evidence against the operation of such a mechanism in neuroblastoma. These inhibitor effects do not eliminate the possibility that the characteristic properties of cholinergic choline transport emerge when a universal uptake system is coupled to choline acetyltransferase. If coupling were absent in neuroblastoma cells, choline kinase and acetyltransferase could compete for their common substrate.

Although the absence of the brain synaptosomal high affinity choline transport system may simply be correlated with the absence of synapses in neuroblastoma cultures15, there are other explanations of our data. In particular, synthesis of choline acetyltransferase may not be an expression of more generalised cholinergic potential of the NS20 clone. This view is supported by the existence of acetylcholine receptors in neuroblastoma cells16, a finding that suggests a postganglionic origin for the cells and seems incompatible with the concomitant presence of cholinergic synaptic structures. Nevertheless, the generation of a high affinity uptake system possessing such properties as preferential synthesis of acetylcholine and sensitivity to appropriate inhibitors could provide a convenient biochemical tool for investigating the differentiation of neuroblastoma cells in vitro.

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# Binding of interferon to gangliosides

RECENT evidence suggests that the antiviral action of interferon is triggered by interaction with the cellular membrane. Mouse interferon covalently bound to Sepharose beads (IF-Sepharose) retains its antiviral potency and only direct contact with these particles produces the antiviral effect<sup>1,2</sup>. Preincubation of mouse L cells with *Phaseolus vulgaris* phytohaemagglutinin (PHA) blocks interferon action3. The inhibitory action of PHA can be almost completely reversed by washing PHA-treated cells with fetuin, a glycoprotein of high affinity for this plant lectin3. These data suggest that membrane sites interacting with interferon are carbohydrate-containing molecules that bind to PHA, although other explanations for the inhibitory action of PHA based on nonspecific steric or charge effects might be possible. To substantiate further that glycoside-containing membrane components bind to interferon, we have investigated the effect of gangliosides on interferon binding and

As Table 1 shows, preincubation of IF-Sepharose with a ganglioside mixture from bovine brain completely blocked antiviral activity. When tested individually, mono- as well as diand trisialogangliosides were inhibitory. The concentration in the preincubation mixture resulting in maximal inhibition of antiviral activity was lowest for  $G_{M2}$  and  $G_{T1}$ , suggesting somewhat stronger binding of these two gangliosides. Thinlayer chromatography on silica plates in chloroform-methanolwater (65:45:9) followed by resorcinol spray4 revealed one band for  $G_{M2}$ , but showed that commercial  $G_{M1}$ ,  $G_{D1\,a}$  and  $G_{T1}$ were somewhat contaminated with other gangliosides although free of  $G_{M2}$ . In separate experiments using  $G_{M1}$ ,  $G_{D1a}$  and  $G_{T1}$ which were 82-87% pure, essentially similar results were obtained, though  $G_{M1}$  and  $G_{D1a}$  appeared slightly less inhibitory.  $G_{M3}$ , a mixture of the acetyl and the glycolyl neuraminic acid derivatives, was only tested at one concentration because of the small amount available. Its inhibitory action appeared slightly less than that of  $G_{M2}$  and  $G_{T1}$ , but more than that of the other two gangliosides. In our determinations of viral yield only differences greater than twofold were considered significant. Therefore, although Table 1 compares µg quantities of gangliosides instead of µmol, the accuracy of the assay does not allow conclusions based on molecular weight differences of individual gangliosides. Inhibition of IF-Sepharose by individual gangliosides was the same regardless of whether preincubation was carried out for 1 h or 18 h. When the temperature of the preincubation mixture was varied, however, there was a consistent increase of the inhibitory effect of gangliosides with increasing temperatures. Thus, EMC yield by plaque-forming units (PFU) in response to IF-Sepharose preincubated with mixed gangliosides at 4° C was one-third of that found after preincubation at 37°C. Washing ganglioside-treated IF-Sepharose with PHA solutions partially reversed ganglioside inhibition, suggesting that PHA and interferon have common binding sites (Table 2). It was not possible completely to restore antiviral activity, however, indicating that under the conditions

Table 1 Inhibition of interferon-Sepharose by gangliosides

Ganglioside in preincubation mixture	Viral yield after preincubation of IF-Sepharose with different amounts of ganglioside (µg)						
	400	200	80	40 `	20	0	
Ganglioside mixture	4,096 (4,096)	ND	512	ND	64	64 (4,096)	
$G_{T_1}$	ND	ND	2,048	256	64	32/64 (2,048)	
G <sub>D18</sub> G <sub>M1</sub>	ND	1.024	256	128	64	32/64 (2,048)	
$G_{M1}$	ND	2,048	256	64	32	32/64 (2,048)	
$G_{M_2}$	ND	2.048	2.048	256	64	32/64 (2,048)	
G <sub>м 3</sub>	ND	ND	256	ND	ND	4/8 (512)	

Interferon covalently bound to Sepharose (IF-Sepharose), prepared as described previously<sup>1,2</sup>, was treated with gangliosides by incubating 5 × 10<sup>4</sup> beads with 0.4 ml ganglioside solution in PBS for 75 min at 25° C. The beads were then washed once with 4 ml PBS and suspended in 1 ml of Eagle's minimal essential medium plus Hanks salts without serum (MEM). The gangliosidemixture was obtained from bovine brain (Sigma, type III). Individual gangliosides were obtained from Supelco Inc. Mouse L cells were cultivated in 35 mm plastic dishes (5 × 10<sup>5</sup> cells per dish) in MEM, containing 10% calf serum. After removal of medium the cells were incubated for 5 h at 37° C with a 1-ml suspension of IF-Sepharose or control Sepharose 4-B in MEM at a ratio of one bead per 10 cells. After removal of the beads, the cells were challenged with encephalomyocarditis virus (EMC) at a multiplicity of infection of 0.1. Viral yield was determined after 16 h of incubation at 37° C by haemagglutination of human red blood cells of type 0 in serial twofold dilutions of virus suspensions 10. The numbers represent the reciprocal of the highest dilution that showed haemagglutination. The individual gangliosides were designated according to Svennerholm 11. Numbers in brackets represent experiments in which control Sepharose 4-B was used instead of IF-Sepharose. ND, Not done.

Table 2 Effect of PHA on ganglioside inhibition of interferon-Sephanose

	interferon-Sepharose	e	
Ganglioside in preincubation mixture	Viral yield without ganglioside pretreat- ment of IF-Sepharose	ganglion ment of	yield after side pretreat- IF-Sepharose A After PHA
Ganglioside	,	octore 111	A Allel FIIA
mixture (400 μg)	64	4.096	512/1,024
$G_{M2}$ (80 µg)	32/64	2,048	512
$G_{M1}$ (200 µg)	32/64	2,048	512
$G_{D1a}$ (200 µg)	32/64	1,024	512
$G_{T1}$ (80 µg)	32/64	2,048	512

After treatment of IF-Sepharose beads with gangliosides as described under Table 1, they were suspended in 0.3 ml of PHA (phyto-haemagglutinin from *Phaseolus vulgaris*, M form, Grand Island Biological Co.) at a protein concentration of 4.1 mg ml<sup>-1</sup>. After incubation for 60 min at 25° C the lectin solution was removed; the beads were washed once with 4 ml PBS, and suspended in 1 ml MEM without serum. Further experimental details were as described under Table 1.

used the affinity of gangliosides for interferon is greater than for PHA. Preincubation of L cells with gangliosides did not result in stimulation of the antiviral effect of interferon, as might be expected if gangliosides became incorporated into the cell membrane to produce additional receptor sites. Such stimulation of activity was observed with cholera toxin $^5$ , which has been shown to bind strongly to  $G_{M1}$  (ref. 6).

Incubation of interferon solutions with gangliosides bound covalently to a Sepharose-polylysine copolymer as described by Cuatrecasas resulted in complete adsorption of antiviral activity to these beads (Table 3). In contrast, under identical conditions control beads that only contained covalently bound polylysine did not bind interferon to any great extent. Binding of interferon to Sepharose-bound gangliosides was prevented by preincubation of these beads with PHA, in accord with previous results suggesting that PHA and interferon bind to common constituents on the cell membrane3. Washing PHAtreated ganglioside beads with fetuin, a protein of known affinity for PHA which reversed PHA inhibition of interferon action3, failed to restore measurable interferon binding to ganglioside beads, even at a concentration of 60 mg ml<sup>-1</sup>. It appears, therefore, that PHA has a higher affinity for Sepharosebound gangliosides than for fetuin in solution. Ganglioside-Sepharose beads also completely inhibited the action of soluble interferon, as Table 4 shows.

Gangliosides are common constituents of cellular membranes. The occurrence of disialoganglioside  $(G_{D1})$  and haematoside  $(G_{M3})$  in the cell membrane of L cells has been reported. Inhibition of mouse interferon action by preincubation of

Table 3 Binding of interferon to ga	anglioside-Sepharose beads
Interferon preincubated with	Interferon titre in supernatant
Nothing	1,024
Control beads	1,024
Control beads	-
Pretreated with PHA	1,024
Ganglioside beads	< 16
Ganglioside beads	
Pretreated with PHA	1,024

Mouse interferon was prepared by induction of mouse L cells with ultraviolet-irradiated Newcastle disease virus in serum-free MEM plus Earle's salts as described before<sup>12</sup>. Crude interferon solutions were concentrated tenfold by pressure dialysis at pH 3 and used as such after changing the pH to 7. They contained 0.2 mg ml<sup>-1</sup> protein<sup>13</sup>. Polylysine (molecular weight 30,000, Sigma) was coupled to CNBr-activated Sepharose (Pharmacia) as described by Sica et al.<sup>14</sup>. Mixed gangliosides from bovine brain (type III, Sigma) were attached to polylysine-Sepharose as described by Cuatrecasas7. Covalently bound gangliosides correspond to 0.15 mg ml<sup>-1</sup> packed beads, as judged from the amount of carbohydrate in the soluble portion of the reaction mixtures after completion of the reaction15. In the experiments with PHA, 0.2 ml of packed beads was incubated for 30 min with 0.5 ml lectin solution (4.1 mg ml<sup>-1</sup> protein, see Table 2) for 30 min at 25° C. They were then washed twice with 1 ml of 1 M NaCl and twice with 1 ml of PBS. In each of the experiments listed 0.2 ml packed beads (washed twice with 1 ml of NaCl and twice with 1 ml of PBS immediately before use) were suspended in 0.5 ml of interferon solution and incubated for 45 min at 25° C with occasional stirring. The supernatant of the beads was collected, and the beads were washed twice with 1 ml of 1 M NaCl and twice with 1 ml of PBS. Original supernatant and washings were combined and the interferon titre was measured by determining the cytopathic effect in serial twofold dilutions on mouse L cells after infection with vesicular stomatitis virus (VSV). Control beads represent polylysine-containing beads before ganglioside attachment. In the control experiment without beads interferon solution was incubated alone and diluted with corresponding amounts of 1 M NaCl and PBS. The numbers represent the reciprocal of the dilution of combined supernatants and washings showing a cytopathic effect of 50%.

L cells with PHA suggests involvement of carbohydrate-containing cell membrane constituents in interferon binding and/or action<sup>3</sup>. Our data show binding of interferon to gangliosides and inhibition of interferon action by exogenous gangliosides. They also demonstrate that inhibition by and binding to gangliosides is partially or completely reversed by PHA, in accord with our previous results<sup>3</sup>. Binding to gangliosides has been shown for certain toxins, like cholera toxin and tetanus toxin, for hormones like seretonin<sup>6</sup>, and for viruses like Sendai<sup>9</sup>. Our data suggest that binding to gangliosides might also play a role in interferon action *in vivo*.

Table 4 Inhibition of interferon activity by ganglioside-Sepharose beads

A. A	Bead to	Viral yield (PFU m <sup>-1</sup> ×10 <sup>-6</sup> )			
Beads used	cell ratio			Interferon adde	
		I "	II	I	II
None		1,200	740	5	3
Control beads	<b>f</b> :8	ND	340	ND	2
Ganglioside beads	1:8	ND	430	ND	170
Control beads	1:10	800	ND	5	2
Ganglioside beads	1:10	800	ND	200	170
Control beads	1:20	ND	ND	ND	2
Ganglioside beads	1:20	ND	ND	ND	32

Polylysine-Sepharose (control) or ganglioside-Sepharose beads prepared as described under Table 3 were suspended in 1 ml of MEM without serum and layered on to mouse L cells in 35 mm plastic Petri dishes (5×10<sup>5</sup> cells per dish). Mouse interferon prepared as described under Table 3 was added and the cells were incubated at 37° C for 24 h. After removal of medium (derivatised Sepharose beads remained tightly attached to the cells) the cells were infected with VSV at a multiplicity of infection of 0.1 and further incubated for 16 h at 37° C. Viral yield was measured directly by determination of plaqueforming units (PFU). I and II represent two different sets of experiments. ND, Not done.

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#### Evidence for ATP action on the cell surface

It seems that Mg2+-ATPase (EC 3.6.1.3) and 5'-nucleotidase (EC 3.1.3.5) are prevalent plasma membrane marker enzymes in eukaryotic cells1. Our studies on the 'sidedness' of plasma membrane macromolecules suggested that the two enzymes function as ecto-enzymes<sup>2</sup>, although the substrates presumably were not present on the cell surface. We assumed that the substrates were present, but obscured within the intercellular spaces and by rapid metabolism. The specificity of the ecto-ATPase is low but we suspected that translocated cytoplasmic ATP might be a primary substrate.

We have recently obtained evidence in tissue cultures for apparent ATP translocation from the cytoplasm into the medium (unpublished data). Reports by McIlwain et al. that adenosine, and to a lesser extent adenylates, were released from electrically stimulated brain slices support this possibility<sup>3-5</sup>. Pull and McIlwain had speculated4 that ATP was the source of the released adenosine. We postulated that the translocation of cytoplasmic ATP might be part of a common physiological phenomenon. During a study of the effects of ATP application to the membrane surface I have now found that the addition of ATP to monolayer cultures of mammalian cells produces a biphasic change in membrane permeability. In situ this event may be initiated by the translocation of cytoplasmic ATP and terminated by ecto-nucleotidephosphoesterhydrolases.

Changes in membrane permeability were measured by determining the extrusion of isotopic ions from pulse labelled monolayer tissue cultures. I shall discuss the permeation of isotopic species because measurements of net fluxes have not been made yet. The basic methodology was briefly as follows. Established tissue culture cell lines were grown in 250 ml Falcon flasks in the appropriate medium. When the cultures reached confluency they were exposed for 2 h to 5 ml of medium containing <sup>32</sup>P<sub>1</sub>, <sup>42</sup>K+ or other isotopic compounds. After removal of the isotopic medium by several brief rinses, the cultures were superfused with 4 ml of serum-free medium at 37° C in an air/ CO<sub>2</sub> atmosphere (95/5, v/v). During incubation the flasks were rotated horizontally once every 150 s for 6 s at 50 r.p.m. to provide stirring action. Extrusion of isotopic species was measured by sampling the medium at intervals and by scintillation counting. Protein content of the cultures was determined in NaOH digests according to Lowry et al.6.

Using <sup>32</sup>P pulse labelled cultures of mouse fibroblasts (L929). HeLa cells (human choriocarcinoma) or KB cells (human carcinoma of the nasopharynx) I found that 32P efflux into the medium increased significantly when ATP reached about 5×10<sup>-4</sup> M. In monolayer cultures of neonatal Syrian hamster astrocytes, the effect of ATP is particularly evident and typical dose responses are illustrated in Fig. 1. When the superfusate

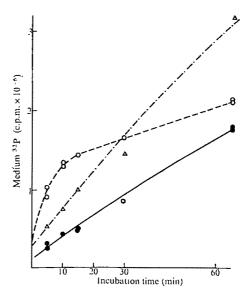


Fig. 1 Effect of medium ATP concentration on 32P extrusion of pulse labelled NN astrocyte cultures. Cultures labelled with <sup>32</sup>P<sub>1</sub> for 2 h, rinsed three times and superfused with Hanks' minimum essential medium containing ATP as indicated. Curves show cumulative  $^{32}P$  in medium.  $\triangle$ ,  $5 \times 10^{-3}$  M;  $\bigcirc$ ,  $1.2 \times 10^{-4}$  M; control.

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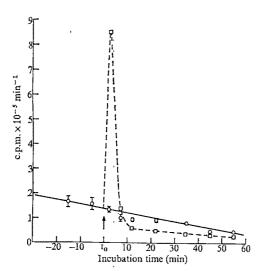


Fig. 2 Rate of extrusion of  $^{42}K^+$  from NN astrocyte cultures after  $6.25 \times 10^{-5}$  M ATP in the medium at  $t_0$ . Cultures labelled for 2 h with 5 mM  $^{42}K$ Cl, rinsed and superfused with Hanks' minimum essential medium. Average of duplicate experiments.

ATP concentration was 5 mM, <sup>32</sup>P extrusion into (and accumulation in) the superfusate proceeded at an accelerated steady rate. At lower concentrations initial efflux was even faster but soon decreased below the rates of the controls.

Testing of other compounds showed that a signal of the sort given by ATP could only be elicited with nucleotide triphosphates, and UTP, GTP and ITP were about equally effective. This was reminiscent of the ecto-ATPases with a similar lack of specificity towards nucleosidetriphosphates<sup>2</sup>. Tests with other ATP metabolites revealed that adenosine (but not uridine, guanosine or inosine) had an effect opposite to that of ATP. Adenosine decreased the extrusion rate of <sup>32</sup>P from labelled cultures and inhibited ATP-induced acceleration of <sup>32</sup>P extrusion at 10<sup>-6</sup> M; the effect seems to apply specifically to the transfer of <sup>32</sup>P.

A typical experiment with 42K+ pulse labelled NN astrocytes is illustrated in Fig. 2. After measuring the extrusion rate of  $^{42}$ K+ for 30 min,  $6.2 \times 10^{-5}$  M ATP was applied at  $t_0$ , resulting in an immediate fivefold increase in 42K+ efflux rate. Shortly thereafter apparent flux decreased below control levels, producing a biphasic signal in response to ATP application. A similar, apparently excitatory-inhibitory, response to ATP was observed by Harary and Farley in cultured beating rat heart cells7. At appropriate doses the effect of ATP on pulse labelled cells was observed in the N-18 neuroblastoma, HeLa cells, KB cells, glioblastoma GL-26 and L-929 fibroblasts. Different cell lines had significantly different dose responses to added ATP. The NN astrocytes responded to  $5 \times 10^{-7}$  M ATP, the N-18 neuroblastoma with not less than  $5 \times 10^{-4}$  M (Fig. 3) and sea urchin eggs, human erythrocytes and a human oligodendroglioma failed to respond.

The effect of ATP on ion permeation was temperature dependent. In the absence of Ca2+ no effect was obtained or it was markedly diminished. In the NN astrocytes 2 mM Ca<sup>2+</sup> was optimal and higher calcium concentrations inhibited the signal produced by ATP. Addition of 10% foetal calf serum to the superfusate did not inhibit the ATP effect. Attempts to demonstrate the ATP effect in cultures pulse labelled with 22Na+ or 36Cl- were not successful because these monolayer cultures are too clumsy a tool for studies which might involve time constants of the orders found in neurochemical transmission. Mostly using NN astrocyte cultures I have found that the ATP signal could not be elicited by acetylcholine, noradrenaline, caffeine, metrazol, inorganic pyrophosphate, phosphocreatin, 3', 5' cyclic AMP or strychnine. The following potential inhibitors proved inactive: ouabain, N-ethylmaleimide, colchicine, avidin, p-hydroxymercuribenzoate, ethylmorphine and theophylline. Oligomycin at 10 µg

ml<sup>-1</sup> inhibited ecto-ATPase activity by about 50%. The same concentration partially inhibited the ATP signal in some cultures, but it also effected a moderate increase in extrusion rates by itself. This again suggests an interrelationship between ecto-ATPase and the ATP-induced increase in permeation rates

Earlier observations may support the conclusion that these data reveal a physiological response. The apparent translocation of ATP from the cytoplasm into the environment has been observed under other circumstances. Aboud et al.8 found a considerable increase in 32P efflux from labelled frog spina root nerves and sartorius muscle during electrical excitation. They concluded that the outflux of acid-soluble phosphates, particularly <sup>32</sup>P<sub>1</sub> and AT<sup>32</sup>P, was somehow related to physicochemical changes of membrane depolarisation. In analogous experiments Silinsky and Hubbard9 detected ATP release from phrenic nerve-hemidiaphragm by the bioluminescence assay. Boyd and Forrester<sup>10</sup> observed release of ATP from frog skeletal muscle in vitro and it was also shown that ATP was released from exercising human forearm muscle11,12. An alternative explanation of the occurrence of extracellular ATP was advanced by Agren et al. 18,14 who observed apparent extracellular ATP synthesis by yeast cells and by various normal and neoplastic tissue cultures. The release of purine nucleotides-(by implication the translocation of ATP) is one of the principal elements of a purinergic autonomic nervous system which has been reviewed in detail by Burnstock<sup>15</sup>.

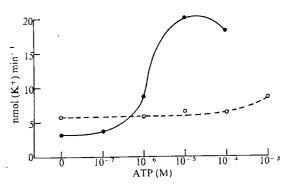


Fig. 3 Rates of extrusion of <sup>42</sup>K from pulse labelled NN astrocyte (●) and N-18 mouse neuroblastoma (○) cultures as a function of ATP concentrations at t<sub>0</sub>. Rates as efflux of <sup>42</sup>K min<sup>-1</sup> during the first 5 min after ATP addition.

The significance of my observations is that they offer an explanation for the presence of some enzymes on the surface o the cell. If ATP is translocated following a pertinent stimulus, i might impinge on the membranes of adjacent cells, effecting ar increase in permeability. In addition to the likely efflux of K the respondent cell might extrude some of its cytoplasmic ATI and thereby initiate the spreading or cascading transmission o a signal. Ecto-ATPase and ecto-5'-nucleotidase would then serve to limit or terminate the phase of increased permeability In the microenvironment a change in permeability or excit ability threshold might not be affected by the action of fre-ATP on the cell surface but by a membrane-bound species Conceivably, the entire process (presumably of altering th plasma membrane structure briefly) requires a phosphoryla tion reaction on the outer aspect of the membrane. One bio catalyst for this reaction might seem to be an ecto-ATPase.

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# Relationship of α-adrenergic receptors in rat pineal gland to drug-induced stimulation of phospholipid metabolism

NORADRENALINE produces increased uptake of <sup>32</sup>P<sub>i</sub> into acidic phospholipids in the nervous system<sup>1-4</sup>, and neurotransmitters and other stimuli do so in various tissues<sup>5-7</sup>. The rat pineal gland responds to noradrenaline in similar fashion<sup>8-10</sup>. We have shown already that the mechanism by which noradrenaline stimulates phospholipid metabolism in the pineal gland does not depend on protein synthesis and does not involve either β-adrenergic receptors or cyclic AMP<sup>10</sup>. We also observed that the \u03b3-adrenergic receptor blocking agent propranolol not only failed to counteract the influence of noradrenaline, but itself markedly enhanced the incorporation of <sup>32</sup>P<sub>i</sub> into phospholipids to give a labelling pattern distinct from that produced by noradrenaline<sup>10,11</sup>. We concluded that this action of propranolol was due to its local anaesthetic or membrane perturbing property, since sotalol, a β-adrenergic receptor blocking agent without these other effects<sup>12</sup>, had no influence on phospholipid metabolism. In addition, a series of local anaesthetics yield effects comparable with those of propranolol<sup>13</sup>.

We now have clear evidence for two mechanisms for the stimulation of phospholipid metabolism by pharmacological agents in vitro. Using the rat pineal gland, we found that the effects of noradrenaline and the α-adrenergic agonist phenylephrine on pineal phospholipid metabolism are counteracted by a-adrenergic blocking agents, but that those of propranolol are unaffected.

Pineal glands were removed from ether-anaesthetised, female Charles River rats (150-200 g) and stored in ice-cold incubation medium until used. Individual glands were incubated free-floating in 100 µl of either modified Puck's N-16 medium (Grand Island Biological Co.) or Krebs-Ringer bicarbonate glucose medium containing 10 µCi of 32P<sub>i</sub> (New England Nuclear Corp.) and the desired amounts of drug. After incubation the glands were rinsed in saline and lipids were extracted with chloroform-methanol. Phospholipids were separated by two-dimensional thin-layer chromatography of the washed total lipid extract on Silica Gel H plates (Analtech). The areas which have been shown to correspond to the individual phospholipids under examination were scraped from the plates and counted10,11.

Addition of L-noradrenaline (0.01 mM) increased incorporation of 32Pi into phosphatidylinositol and phosphatidylglycerol by 200-300%, but had no effect on phosphatidylcholine labelling (Table 1). Similar stimulation was obtained with the α-adrenergic agonist phenylephrine (0.01 mM). Neither of the a-adrenergic blocking agents used, phenoxybenzamine (Dibenzyline; Smith, Kline and French) and phentolamine (Regitine; CIBA) at 0.02 mM increased labelling

Table 1 Inhibition of adrenaline- or phenylephrine-induced stimulation of pineal phospholipid metabolism by a-adrenergic receptor blocking agents

	<b>F</b>				
	Incorporation of <sup>32</sup> P <sub>1</sub> into Phosphatidyl- Phosphatidyl- Phosphatidyl-				
Additions	inositol	glycerol	choline		
	(pm	ol per pineal g	land)		
Noradrenaline +	$26.4 \pm 3.5$	1.1+0.1†	$16.8 \pm 4.0$		
Phentolamine					
Noradrenaline					
+	22.4 + 4.0	$0.8 \pm 0.2$	$12.9 \pm 1.6$		
Phenoxybenzamine					
Noradrenaline	$66.3 \pm 5.9 *$	$2.0 \pm 0.1*$	$16.9 \pm 1.6$		
Phenylephrine	_				
+	$23.5 \pm 2.9$	$0.9 \pm 0.1$	$16.7 \pm 4.6$		
Phentolamine					
Phenylephrine					
+	$20.6 \pm 4.0$	$0.9 \pm 0.1$	$11.7 \pm 4.2$		
Phenoxybenzamine					
Phenylephrine	54.2 + 2.8*	$1.7 \pm 0.1*$	$15.3 \pm 1.9$		
Phentolamine	$15.1 \pm 1.9$	$1.1 \pm 0.1 \dagger$	$14.2 \pm 1.2$		
Phenoxybenzamine	$20.1\pm 2.8$	$1.2 \pm 0.1 \dagger$	$15.5 \pm 2.1$		
None	18.6 + 1.6	$0.8 \pm 0.1$	$15.9 \pm 3.1$		

Pineal glands were incubated in modified Puck's medium for 1 h in the presence of the agonists (0.01 mM), the blocking agents (0.02 mM) or both drugs together. Each value represents the mean  $\pm s$ . e. of the mean of at least four pineal glands. Differences from control means (no additions).

of phosphatidylinositol, although some stimulation phentolamine occurred with much larger concentrations of drug. Combinations of agonist and antagonist virtually abolished the response to agonist alone. Both a-receptor blocking drugs prevented the enhanced 32Pi incorporation into phosphatidylinositol and phosphatidylglycerol elicited by either noradrenaline or phenylephrine. These observations strongly indicate the presence of a-adrenergic receptors in rat pineal gland and their involvement in mediating the action of both phenylephrine and noradrenaline, the physiological pineal neurotransmitter, on pineal phospholipid metabolism.

Our finding that propranolol strongly stimulated 32Pi incorporation into lipids10 prompted us to attempt to discriminate between the modes of action of noradrenaline and propranolol. Such a distinction was suggested by different labelling patterns of phospholipids produced by these agents. The differences include stimulation of cytidine diphosphatediglyceride labelling and increased incorporation of isotope into phosphatidylglycerol and phosphatidic acid in the presence of propranolol compared with noradrenaline. We therefore investigated whether an a-adrenergic receptor blocker affected propranolol-induced stimulation of pineal phospholipid metabolism. Although it prevented the effects of noradrenaline or phenylephrine, phenoxybenzamine did not reduce the effects of propranolol (Table 2). The same resistance of propranolol-induced stimulation of pineal phospholipid metabolism to a-adrenergic receptor blockade was observed in experiments with Puck's medium and smaller concentrations of phenoxybenzamine or phentolamine (0.02 mM).

There are already indications that α-adrenergic blocking agents inhibit adrenaline-induced nonspecific stimulation of phospholipid metabolism, obtained with liver slices<sup>14</sup> and fat cell suspensions<sup>15</sup>. Robison et al. speculated that α-adrenergic receptors mediate the effects of catecholamines on phospholipid metabolism16. Later work has shown the ability of phenoxybenzamine or phentolamine to block enhanced phosphatidylinositol labelling from 32Pi, produced by adrenaline, noradrenaline or phenylephrine, in slices of parotid gland17,18 and vas deferens19 as well as in brain in vivo4.

The findings reported here are consistent with these observations. We have demonstrated that the rat pineal gland

<sup>\*</sup>P < 0.001. †P < 0.05.

Table 2 Resistance of propranolol-induced stimulation of pineal phospholipid metabolism to α-adrenergic blockade

Additions	Concentration (mM)	Phosphatidyl- inositol	Phosphatidyl- glycerol	on of <sup>32</sup> P <sub>i</sub> into  CDP-diglyceride pineal gland)	Phosphatidic acid	Phosphatidyl- choline
Propranolol	0.03	141100*	20.04		20105+	90104
+ Phanayyhanzamina	0.06	$14.1 \pm 0.8*$	3.8±0.4*	_	$3.9 \pm 0.5 \ddagger$	8.0 ±0.4
Phenoxybenzamine Propranolol Propranolol	0.00 0.03 0.10	15.0±0.9†	3.4±0.3*		4.4±0.3†	8.3±0.8
+	0.10	18.8 ± 1.2*	8.4±0.3*	3.6±0.5*	$7.2 \pm 0.2 *$	8.7 ±1.6
Phenoxybenzamine Propranolol Phenoxybenzamine None	0.06 0.10 0.06	16.6±2.4† 8.2±0.8 8.5±0.4	$7.5\pm0.9*$ $0.4\pm0.0$ $0.5\pm0.1$	5.0±0.9† 0.2±0.1 0.3±0.0	6.0±0.8† 3.2±0.2 2.5±0.2	7.2 ±0.9 10.4 ±0.6‡ 6.6 ±0.5

Pineal glands were incubated in Krebs-Ringer bicarbonate buffer for 1 h in the presence of DL-propranolol, phenoxybenzamine or both agents together. Each value represents the mean  $\pm$ s. e. of the mean of four pineal glands. Differences from control means (no additions). \*P < 0.001; †P < 0.005; ‡P < 0.02.

possesses  $\alpha$ -adrenergic receptors which mediate the stimulatory effects of noradrenaline on pineal phospholipid metabolism. Earlier the suggestion was made that in the pineal gland "the response to  $\beta$ -adrenergic stimulation can be influenced by an a-adrenergic mechanism", since phentolamine enhanced the effect of noradrenaline on serotonin N-acetyl transferase activity<sup>20</sup>. Until now only β-adrenergic receptors have been definitely shown to be present in the pineal gland<sup>21</sup>.

It has been suggested that  $\alpha$  and  $\beta$ -adrenergic receptors act through a common mechanism, involving opposite effects on the intracellular level of cyclic AMP18. The authors of this hypothesis also cite instances, however, in which α-adrenergic receptor-mediated phenomena are not accompanied by a decrease in cyclic AMP. The pineal gland provides a further instance of stimulation of a-adrenergic receptors which does not depress cyclic AMP, since noradrenaline increases the level of this nucleotide21.

In rat parotid slices α-adrenergic receptors mediate the release of intracellular potassium by adrenaline, a process which requires Ca2+ and energy22 and which can similarly occur without changes in the cyclic AMP level of the cell. These observations suggest that the physiologically significant aspect of the stimulation of the acidic phospholipid metabolism brought about by catecholamines involves a relationship between the a-adrenergic receptor and ion movements. The pineal gland may be a suitable model system for the study of these interrelationships.

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# Octopamine receptors on Aplysia neurones mediate hyperpolarisation by increasing membrane conductance

OCTOPAMINE is a phenylethylamine synthesised by β-hydroxylation of tyramine and differs from noradrenaline in lacking one hydroxyl group on the phenyl ring. Although Aplysia nervous tissue, like that of most molluscs, contains little or no noradrenaline<sup>1,2</sup>, it has between 0.20 and 1.85 pmol octopamine per mg of tissue in the various ganglia. Individual identified neurone also contain detectable octopamine and in neurone R14 i reaches  $1.5 \times 10^{-4}$  M (ref. 2). Although octopamine-activated adenyl cyclases have been found in Aplysia nervous tissue3 and insects4.5, there has been no electrophysiological demonstration of specific octopamine receptors. We now report receptors in the nervous system of Aplysia that are most sensitive to octo pamine and mediate a hyperpolarising conductance-increase response. This provides further evidence that octopamine car function as a neurotransmitter.

Abdominal, cerebral, pleural and pedal ganglia of Aplysia californica or Aplysia dactylomela were removed and pinned to Sylgard (Dow Corning) in a lucite chamber. The connective tissue capsule was slit with a razor blade to expose the cel bodies. Neurones were penetrated with a double barrelled glass micropipette filled with 2 M potassium acetate and recording was performed as before. Constant current pulses and/or current were applied through one barrel of the electrode as needed, while recording was made through the other. The

ganglia were constantly perfused with high Mg<sup>2+</sup> (150 mM) seawater, which blocks transmitter release from nerve terminals. Consequently, responses to iontophoretic drug application may be considered to be direct effects.

Octopamine and related compounds were dissolved in distilled water at concentrations of 1 M (pH 3-4) and each compound was placed in one barrel of a five barrelled iontophoretic electrode which was bumped at the tip to give a resistance of 2-5 M $\Omega$  for each barrel. The controller for the iontophoretic current was designed to pass a constant charge between 0 and 1,000 nC (J. Willis, in preparation). Thus the duration of the pulse was varied automatically to ensure that the same total charge was passed from each barrel, making possible a meaningful comparison of the response of one cell to several putative transmitters. When an octopamine response was found, the sensitivity of that site was tested with all other drugs in the iontophoretic electrode.

Responses to octopamine were recorded in cells from all ganglia studied but were obtained in only a small percentage of neurones. They were most common in cerebral ganglia, which also contain the most octopamine<sup>2</sup>. In all cases receptors for octopamine, like those for dopamine<sup>7</sup>, were not on the soma but rather in the neuropile. This may have led to an underestimation of the percentage of neurones containing octopamine receptors since it was necessary to search blindly for sensitive areas in the neuropile.

Figure 1 illustrates the response of a neurone in the cerebral ganglion to octopamine. A pulse of 1,000 nC octopamine resulted in a slow hyperpolarisation peaking at about 12 S. Pulses of 2,000 nC noradrenaline and phenylethanolamine, which differ from octopamine in having one additional and one less hydroxyl groups respectively, gave small responses. Dopamine was ineffective in exciting this receptor.

Figure 2 illustrates another experiment in which a pulse of 500 nC octopamine gave a 5 mV hyperpolarisation (a). When constant pulses (10<sup>-10</sup> A) were applied (b), the amplitude of the voltage deflection during the response was reduced. This reduction was not a result of nonlinear current-voltage relationships (not illustrated), and thus reflects an increased conductance to some ion, such as K<sup>+</sup> or Cl<sup>-</sup>, which has an equilibrium potential more negative than the resting potential. In (c) the ganglion was perfused with seawater in which all Cl<sup>-</sup> was replaced with acetate, which is relatively impermeable. In such a solution the equilibrium potential for Cl<sup>-</sup> shifts and becomes depolarising, as is the case for Cl<sup>-</sup> conductance responses to acetylcholine<sup>8</sup>. This response did not change, how-

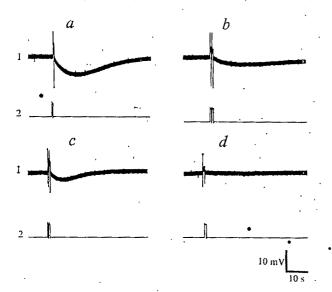


Fig. 1 Response of an unidentified neurone in the cerebral ganglion to octopamine (a), phenylethanolamine (b), noradrenaline (c) and dopamine (d). Trace 2 shows the duration of the iontophoretic pulse. Each pulse passed 1,000 nC. Double pulses were given for phenylethanolamine and noradrenaline.

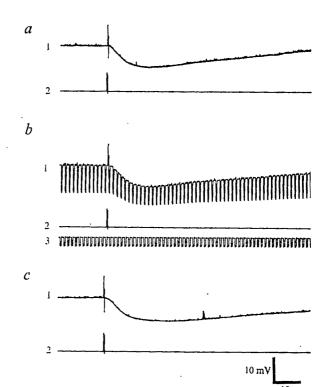


Fig. 2 Responses to 500 nC octopamine from an experiment on another unidentified neurone in the cerebral ganglion (a). For trace (b) constant current pulses (b-3) were applied during the application of octopamine. The smaller amplitude of the voltage deflection during the responses indicates a conductance increase since the neurone had a linear current-voltage relationship over this range of potential. For trace (c) the ganglion was perfused with Cl-free (acetate) seawater. Membrane potential was adjusted to control levels by applied depolarising current. The fact that the response is as large as control indicates that it is not a result of a Cl-conductance increase.

ever, and thus it is likely that it results from an increased K<sup>+</sup> conductance. It was not possible to reverse most of these responses by applied hyperpolarisation. This is probably because the receptors are all on distal processes in the neuropile. A similar difficulty has been reported in reversing K<sup>+</sup> conductance dopamine responses<sup>7</sup>.

The evidence for a neurotransmitter function for octopamine in Aplysia is threefold. (1) It is present in ganglia and is asymmetrically distributed in single, identified neurones. It is present in neurones that contain neither dopamine nor noradrenaline in concentrations as high as 10-4 M (ref. 2). (2) Octopamine receptors are located on a few neurones only and then only in the neuropile, where functional synapses occur. (3) The receptors have a marked specificity for octopamine. Most are also slightly sensitive to noradrenaline and phenylethanolamine, but to a much smaller degree. This suggests that the receptor has an absolute requirement for the β-hydroxyl group and as a result is insensitive to dopamine. Since there is no noradrenaline in Aplysia<sup>1,2</sup>, the responses to it have no functional significance. Phenylethanolamine is present in Aplysia nervous tissue (J. Saavedra, M. Brownstein, D. O. C. and J. Axelrod, unpublished observations) and receptors specifically sensitive to phenylethanolamine are present (our unpublished observations). The sensitivity of the receptors illustrated here is so much greater for octopamine that it seems likely that this is their physiological function. Neurones of the land snail respond to octopamine as well as dopamine and noradrenaline, but none have clearly specific receptors for octopamine only.

Octopamine is present in the nervous and innervated nonnervous tissues of mammals<sup>10</sup> as well as various invertebrates<sup>9,11</sup>. But, as octopamine can replace noradrenaline at its storage sites and be released on nerve stimulation<sup>12,13</sup>, it has been considered to be a false neurotransmitter. Molinoff and Axelrod<sup>14</sup> proposed that, since octopamine is a normal constituent of rat sympathetic nerves, it might normally be released together with noradrenaline. The combination of the demonstration of octopamine in neurones not containing other phenylethylamines and receptors very specific for octopamine argues strongly for a neurotransmitter function of octopamine in Aplysia which is independent of any other structurally related compound.

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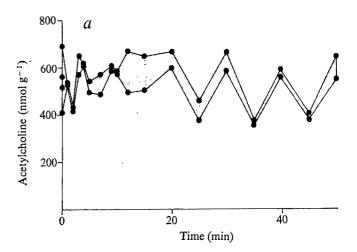
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### Sustained oscillations of acetylcholine during nerve stimulation

THE neurotransmitter acetylcholine (ACh) is synthesised and stored in nerve terminals. Its turnover, rather slow under resting conditions, is greatly accelerated by nervous activity. When action potentials reach the terminals they trigger the release of large amounts of transmitter into the synaptic cleft where ACh can act on postsynaptic receptors and become a substrate for cholinesterase. Then choline and in some cases1 acetate moieties are taken up by the nerve endings and incorporated into new ACh which, in turn, can be rapidly reused. To investigate the turnover of ACh during activity, we have measured acetylcholine at short time intervals after the exposure of the nerve to electrical stimulation at different frequencies. We report here that the concentration of ACh oscillates following the onset of stimulation, and that the period of oscillation is related to the stimulation frequency. The experiments have been performed using the electric organ of Torpedo, a purely cholinergic tissue, which is very homogenous and rich in ACh2-4.

Torpedo marmorata fishes were received alive from the Station de Biologie Marine, Arcachon, France. Most experiments were done on isolated prisms, which are columns of electroplaques representing the functional unit of the organ3. After careful dissection, the prisms were allowed to recover for 1-2 h in an elasmobranch saline medium which maintains the functional and structural properties of the tissue<sup>5</sup>. The prisms were submitted to electric field stimulation for a given time and then rapidly quenched, by one of the following methods. (a) Tissue was plunged into liquid nitrogen, pulverised and allowed to thaw in trichloracetic acid (TCA) which was then extracted with ether, and the ACh determined using the frog rectus muscle bioassay. (b) Saline elasmobranch medium acidified to pH 4 with HCl at 100° C was used. The results obtained with these two methods were identical. The time lag between the end of stimulation and immersion in liquid nitrogen or boiling acidified medium did not exceed 1-2 s.

Figure 1a shows the typical changes in ACh content which occur when the tissue is stimulated. At rest (time of stimulation = 0) there was a rather large dispersion of ACh values, perhaps because the prisms were at different starting conditions. During the first 2s of stimulation, ACh content decreased. After this the first peak was seen, which in all experiments looked sharp and exceeded the mean resting content. The second peak was always lower; at this stage there was again a large dispersion of values. But 20 s from the onset of stimulation,



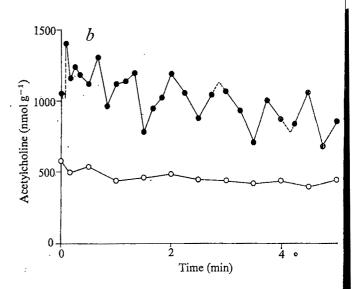


Fig. 1 Dynamic pattern of ACh changes during stimulation. ACh is expressed as nmol per g wet tissue. a, During stimulation at 20 Hz, the period of oscillation is too close to the frequency of sampling to permit a description of the form of the oscilla-tions (results from two experiments on the same *Torpedo*). b, Stimulation at 10 Hz. The bound or vesicular compartment of ACH (O) did not follow the large oscillations of total ACh (O), therefore the changes are largely in the "free" compartment.

the amount of ACh started to show oscillations with a regular period of about 10 s for a stimulation frequency of 20 Hz.

In Fig. 1b the tissue was given a stimulation of 10 s After the initial changes (the first fall, missed here, was found at 2s in another experiment) ACh content oscillated with a period of about 42 s. In this experiment, bound or vesicular ACh was also measured in prisms by immediately homogenising after stimulation and then treating with TCA. In this

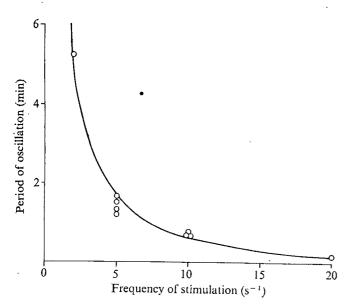


Fig. 2 Period of the ACh oscillations as a function of stimulation frequency. The line is an hyperbola calculated for the best approximation to experimental values.

case, the ACh remaining in the extract is regarded as ACh bound in a compartment associated with synaptic vesicles4,6 whereas ACh hydrolysed during homogenisation (about half of the total amount) is "free" or "available" ACh5,7. Fig. 1b shows that bound ACh did not oscillate during stimulation. Clearly the large variations in the total ACh concerned only the available pool of transmitter. If stimulation is continued, however, a decrease in the vesicular ACh also will occur<sup>5,7,8</sup>.

The variations in ACh content followed the same general pattern at other stimulation frequencies. The period of oscillation approximated a hyperbolic function of the stimulation frequency (Fig. 2). The delay of the first peak obeyed a slightly different relationship, reaching a limit at high stimulation frequencies. At the lower frequencies the shape of the oscillation was far from a sinusoidal wave; the peaks were separated by flat intervals and their rising slope looked smaller than their fall. In a few cases, the electric tissue was also stimulated in vivo through the nerves, the animal being slightly anaesthetised<sup>5</sup>. The results obtained under these conditions were the same as those from excised prisms.

Thus we found that, when driven from the resting to the active state, the electrogenic tissue exhibits a remarkable dynamic pattern. After a somewhat complicated initial phase, the ACh content follows undamped oscillations which are a function of the stimulation frequency. The dispersion of ACh values, rather high at the beginning, decreases during the oscillations. This behaviour could hardly be explained on the basis of a first order kinetic system, even if the conditions were far from equilibrium. On the other hand, sustained oscillations have been predicted and observed in more complicated systems coupled with a flow of matter or energy10-12. The oscillation arises at a key reaction, perhaps as a result of substrate inhibition or product activation of the reaction, although it can be caused in many other ways. For example, diffusion velocities to or through membranes can be important in such phenomena<sup>13,14</sup>. The result is that, when the rates of provision of substrate and removal of product fall between well defined limits, the system follows undamped oscillations, whose period is a function of the rate of provision of substrate. The flux through these systems is subject to sudden variations between two or more steady states, and the initial transition does not occur at the same substrate concentration as the reverse transition.

The resting turnover of ACh in this tissue (ref. 15 and unpublished observations) is about 104 times lower than the maximum speed of net synthesis measured in the present experiments. We think that the mechanisms underlying ACh oscillations are more than a curiosity. They are probably the result of highly regulated metabolic loops and might be a general property of pathways which, when passing from rest to activity, require accelerations of several orders of magnitude.

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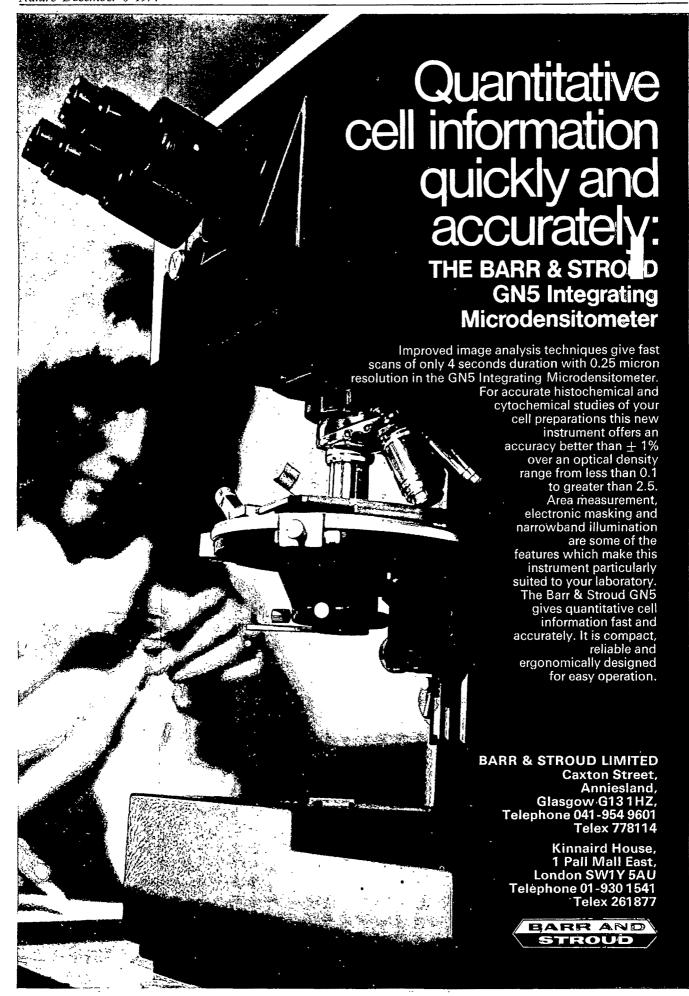
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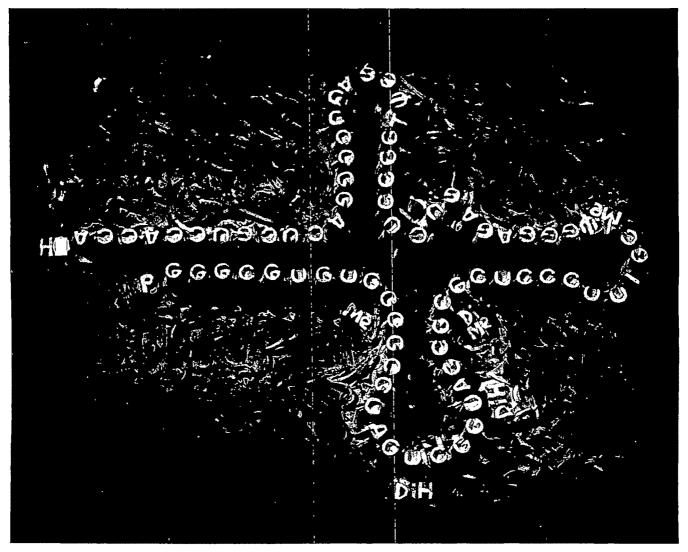
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### Evidence for a direct effect of LRF and TRF on single unit activity in the rostral hypothalamus

Many of the hormones produced by the pituitary and other endocrine glands influence the electrical activity of the brain (for recent references see Dyer1). There is now a growing awareness that some of the hypothalamic releasing factors, which regulate the secretion of hormones from the anterior pituitary gland, may themselves have a similar influence upon the central nervous system<sup>2-4</sup>. The amino acid sequences for both thyrotrophin releasing factor [TRF; (pyro) Glu-His-Pro (NH<sub>2</sub>)]<sup>5,6</sup> and luteinising hormone releasing factor [LRF; (pyro) Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly (NH2)]7 have been fully established and these polypeptides, produced synthetically and of high specific activity, are readily available. The experiments to be described were undertaken to establish whether LRF and/or TRF have a direct effect upon the electrical activity of neurones in the forebrain. Action potentials were recorded from single cells in the hypothalamus and cerebral cortex and LRF and TRF applied to them by microiontophoresis. The sensitivity of some of the neurones to oxytocin (a biologically active peptide of similar size) was also tested.

Female rats were anaesthetised with urethane and prepared for extracellular recording of single unit activity8. Action potentials were recorded through glass micropipettes-filled





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with 4.0 M sodium chloride—with a tip resistance of 5-15M $\Omega$ , which constituted the centre barrel of multi-barrelled electrodes. These were made from glass electrode tubing (H15/10, Jencons) using established techniques and filled with 2.0 M sodium chloride, 0.01 M oxytocin (purified from natural material by Dr B. T. Pickering), 0.01 M LRF (Beckman Ltd and ICI Ltd) and 0.03 M TRF (Beckman Ltd). The overall tip diameter of the electrode was between 1.5 and 3.0 µm and the tip resistance for each pipette containing drugs was in excess of 25 M $\Omega$ . The drugs were retained within the electrodes by currents not exceeding 5 nA and ejected with currents of up to 300 nA, but usually in the range 5-30 nA. (We have not attempted to express the amount of releasing factor applied in terms of mol s<sup>-1</sup> since the conversion from nA requires many assumptions<sup>9</sup> and may produce a very misleading figure.) The polypeptides were only considered to affect the firing rate of a neurone when repeated applications caused at least a 20% change in the discharge rate of the cell over the test period. Routine applications were made for 40 s but some cells were tested for many minutes. Responses which could not be reproduced were excluded and where reproducible responses were obtained, their specificity was checked by application of oxytocin or Na+ at the same current. The apparatus and techniques used were similar to those previously described from this laboratory<sup>10,11</sup>.

Thirty-six single units were fully tested and of these 12 were recorded in the cerebral cortex and 24 in the preoptic and anterior hypothalamic areas (PO/AH). Oxytocin, LRF and TRF did not affect any of the twelve neurones tested in the cerebral cortex. Oxytocin was also without effect on any of the 8 cells tested in PO/AH (Fig. 1). LRF inhibited the spontaneous discharge of 4 out of 12 cells, however, (Fig. 2) and TRF had a similar effect on 7 out of 17 cells (Figs 1 and 2). One cell was excited by TRF and the remaining cells were not affected. Some of the unresponsive cells were subsequently tested by iontophoresing the releasing factors for many minutes but none of the cells exhibited any change in their firing rate which could be attributed to the drug application (Fig. 3). Although not all neurones were fully tested with both LRF and TRF, several units responded to one releasing factor and not the other (Fig. 1). Two cells were inhibited by both LRF and TRF (Fig. 2).

These results show that the releasing factors for thyroid stimulating hormone and luteinising hormone have the

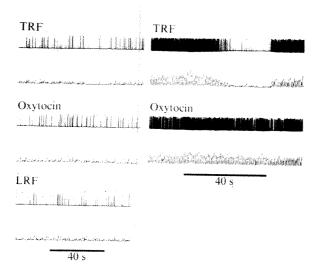


Fig. 1 The polygraph records on the left were obtained from a preoptic neurone which was inhibited by TRF (5 nA) but not LRF or oxytocin (8 nA). The two traces on the right show another unit inhibited by TRF and unaffected by oxytocin (both 7 nA). This second cell was not adequately tested with LRF. (Note, in this and the subsequent figures each action potential is represented by one excursion of the pen (top trace for each test) and an integrated record of this activity is shown in the lower trace. The test period is depicted by the black bar.)

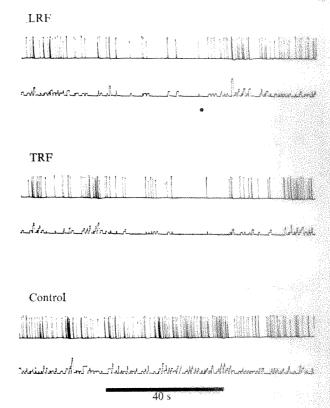


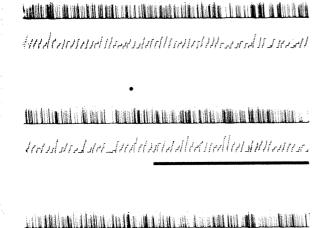
Fig. 2 Polygraph record showing inhibition of a single neurone in PO/AH by both LRF and TRF. The control was Na\* ejected at the same current (8 nA) as the releasing factors.

potential to modify the electrical activity of certain hypothalamic neurones. It is particularly interesting that these releasing factors did not affect all cells and did not influence the firing of any of the neurones tested in the cerebral cortex. It is also important that the firing rate of the responsive cells was neither increased nor diminished by microiontophoretic application of oxytocin at the same current, and that some cells responded to one releasing factor and not the other. Oxytocin has already been shown to excite antidromically identified neurosecretory cells in the paraventricular nucleus of rats and rabbits when applied extracellularly by microiontophoresis<sup>11</sup>. The experiments also showed that the hormone did not affect nonneurosecretory cells in the paraventricular nucleus and we have now extended these findings to show that neurones in the rostral hypothalamus are unresponsive to oxytocin.

The units inhibited by application of releasing factor frequently responded when rather low ejection currents were used (for example about 10 nA) and one cell even showed a substantial reduction in firing rate when the retaining current on the TRF pipette was turned off. On the other hand, many of the unresponsive cells were tested, with the same electrode as responsive cells, at much higher currents and for longer periods of time. Our results show therefore that a discrete population of neurones in the rostral hypothalamus is inhibited by application

of hypothalamic releasing factors.

If releasing factors are important in the control of hypothalamic function two possible routes of access to the neurones can be postulated. In the first place, the releasing factor may be secreted into the portal vessels from which they could be carried through the systemic circulation back to the hypothalamus. This is the conventional understanding of the so-called 'ultra-short loop' feedback system. An alternative mode of application could be trans-synaptic. That is, the releasing factors may be neurotransmitters secreted from branches of peptidergic neurones which do not terminate near the vessels of the primary plexus of the adenohypophysial portal system. In fact, there is some evidence that fibres stained by immunofluorescence for LRF do not always pass to the



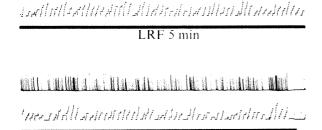




Fig. 3 Continuous polygraph record to show prolonged application of LRF (13 nA) on to a neurone which did not respond to the standard test period of 40 s. Note that even after 5 min the firing rate was unchanged.

median eminence. Such fibres have been described passing from the hypothalamus towards the mesencephalon and mammillary bodies as well as in the amygdala12.

A neurotransmitter function for releasing factors, in addition to their classical role, could provide a possible mechanism for the coordination of different aspects of hypothalamic function. There is no theoretical reason why neurones which secrete releasing factors into the hypophysial portal vessels may not have collateral branches which synapse on to other hypothalamic cells. In this way a single neurone or group of neurones in the hypothalamus could influence both the behaviour of the animal and secretion of its pituitary hormones.

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### In vitro enhancement of hepatic microsomal biphenyl 2-hydroxylation by carcinogens

Many natural and synthetic chemicals in the environment may be carcinogenic, but it is clearly impracticable to assess the potential hazard from all such compounds by existing in vivo methods. Simple preliminary screening procedures are therefore urgently needed to identify the compounds most likely to be hazardous and allow detailed study of these suspected carcinogens. A series of fundamentally different test systems involving a range of biological materials will probably be necessary. As most carcinogens seem to act through active metabolites produced by the enzymes of mammalian endoplasmic reticulum<sup>1</sup>, any test system probably requires the presence of these enzymes and the cofactor NADPH<sub>2</sub>.

Assessing the damage to the DNA of growing bacteria which is produced by carcinogens in the presence of mammalian drug metabolising enzymes—whether in vivo2 (host mediated assay) or in vitro using liver cell fractions (Ames test)3—seems to be the most successful approach so far. The estimation of changes inflicted on the endoplasmic reticulum, however, (such as the in vitro displacement of ribosomes (degranulation) of the liver endoplasmic reticulum by carcinogens in the presence of NADPH<sub>2</sub> (ref. 4)) and the in vivo effects of carcinogens on drug metabolising enzymes<sup>5</sup> are promising alternatives.

Here we report that the in vitro incubation of some chemically dissimilar compounds which are carcinogenic to rat hepatic microsomes in the presence of NADPH, produces a selective increase in biphenyl 2-hydroxylation, but has no significant effect on biphenyl 4-hydroxylation. Non-carcinogens do not enhance either hydroxylase.

The incubation system was as follows: Test compounds (carcinogens and non-carcinogens) in either groundnut oil, or in 1.15% KCl plus 1% Tween 80, were added to hepatic microsomes in buffer solution prepared from male adult Wistar rats (final protein concentration of 2 mg ml-1 in the presence of an NADPH-regenerating system of glucose-6-phosphate (25 mM), NADP (500 nmol), glucose-6-phosphate dehydrogenase (2 unit ml-1) and magnesium sulphate (50 mM) and the system preincubated for 10 min at 37° C in a shaking water bath at 100 cycles min<sup>-1</sup>. Biphenyl (13 mM in 1.15% KCl containing 1.5% Tween 80) was then added and the incubation continued for a further 5 min. The reaction was stopped by the addition of 1 ml of M HCl and the 2- and 4-hydroxybiphenyls thus formed were extracted and determined fluorimetrically6. In the controls, the test substance or biphenyl was either left out or added after the end of the reaction.

Preincubation of rat microsomes with a wide range of carcinogens such as 3, 4-benz(a)pyrene, aflatoxin B1, 2-acetamidofluorene and dimethylnitrosamine caused a significant enhancement of biphenyl 2-hydroxylation. In contrast, noncarcinogenic compounds, such as salicylate, phenobarbitone and 1,2,3,4-dibenz(a)pyrene, produced no significant effect on the biphenyl 2-hydroxylase. Neither class of compound produced any significant changes in biphenyl 4-hydroxylation (Table 1). Interestingly, carbon tetrachloride, a hepatotoxic agent and a questionable carcinogen, produced only a slight enhancement of biphenyl 2-hydroxylation. It is apparent that the enhancement of the hepatic biphenyl 2-hydroxylase activity is not confined solely to hepatocarcinogens for β-naphthylamine, benz(a)pyrene and methylcholanthrene are all effective.

This is probably because our in vitro system has optimal conditions for the metabolism of most carcinogens, which produce their active metabolites through microsomal metabolism, a situation which clearly does not pertain in vivo. The effect on biphenyl 2-hydroxylase seems to be relatively specific because other drug metabolising enzymes, such as p-nitroanisole demethylase and aniline hydroxylase, were either unaffected or slightly inhibited following preincubation with carcinogens and non-carcinogens (F.McP., J.W.B., and D.V.P., unpublished). Similar results have been found using hamster liver microsomes. The possibility of the carcinogen provoking an allosteric conversion between the two hydroxylases seems unlikely in view of the apparent lack of correlation between changes invoked in biphenyl 2- and 4-hydroxylation.

Table 1 The effects of preincubation with carcinogenic compounds on the biphenyl 2-hydroxylase and biphenyl 4-hydroxylase of rat hepatic microsomes

Test compound*	Carcino- genicity	lative to co Biphenyl 2-	n activity, re- ntrol values Biphenyl 4- hydroxylase
3,4-Benz(a)pyrene 22-Methylcholanthrene 20-Methylcholanthrene 2-Acetamidofluorene Dimethylnitrosoamine Aflatoxin B <sub>1</sub> Safrole Piperonyl butoxide Piperonyl sulphoxide Isosafrole a Napthylamine β Napthylamine Carbon tetrachloride Phenobarbital Nikethamide 1,2,3,4-Dibenz(a)pyrene Hexobarbital Aniline Salicylic acid Piperidine Piperanyl Eugenol EDTA	+++++++++++++++++++++++++++++++++++++++	$\begin{array}{c} +252\pm23\dagger\\ +243\pm19\dagger\\ +125\pm11\dagger\\ +74\pm9\dagger\\ +98\pm11\dagger\\ +82\pm7\dagger\\ +82\pm7\dagger\\ +62\pm5\dagger\\ +62\pm9\dagger\\ +47\pm3\dagger\\ +17\pm12\\ +198\pm14\dagger\\ +34\pm8\dagger\\ +16\pm9\\ +10\pm12\\ +8\pm9\\ +11\pm6\\ -12\pm7\\ +3\pm5\\ +7\pm6\\ 0\pm7\\ +3\pm5\\ +7\pm6\\ 0\pm7\\ +3\pm6\\ 0\pm7\\ 144\\ 0\pm7\\ 144\\ 144\\ 144\\ 144\\ 144\\ 144\\ 144\\ 14$	$\begin{array}{c} -9\pm 6 \\ +8\pm 9 \\ -4\pm 7 \\ -9\pm 7 \\ -18\pm 5 \\ +2\pm 6 \\ -34\pm 6\dagger \\ -31\pm 4\dagger \\ -28\pm 7\dagger \\ -34\pm 3\dagger \\ -4\pm 3 \\ +3\pm 6 \\ -39\pm 6\dagger \\ -8\pm 6 \\ -12\pm 4 \\ +4\pm 7 \\ +2\pm 5 \\ -5\pm 3 \\ +4\pm 7 \\ -3\pm 7 \\ 0\pm 8 \\ -37\pm 3\dagger \end{array}$

Results ± s.e.m. for six animals

Our observations may be the result of a direct effect of the carcinogen itself or may be mediated by the formation of an active metabolite. It is generally accepted that most carcinogens produce their in vivo effects through their metabolism to an active electrophilic or free radical intermediate7-10, probably effected by the NADPH-cytochrome P-450-dependent microsomal hydroxylase system<sup>1</sup>. Inhibitors of cytochrome P-450 could not be used directly to establish whether formation of an active metabolite from the carcinogen was a prerequisite for the enhancement of biphenyl 2-hydroxylase because they would also interfere with biphenyl metabolism.

Evidence that these carcinogens require metabolism before producing their effects, however, was obtained by examining the cofactor requirements and the incubation time dependency of the increased biphenyl 2-hydroxylase activity. A characteristic of the biphenyl 2-hydroxylase is that, unlike most drug oxidation reactions (including biphenyl 4-hydroxylation) it is not totally dependent on NADPH being able to utilise NADH instead. Thus, on preincubation of hepatic microsomes with safrole in the presence of NADH instead of a NADPHregenerating system, no enhancement of biphenyl 2-hydroxylation was observed although control values of this enzyme activity were unaffected by the change of cofactor. Presumably the explanation of this finding is that in the presence of NADH, safrole was not converted to an active metabolite. The length of preincubation time was also found to determine the degree of enhancement of the biphenyl 2-hydroxylase activity, optimal stimulation for safrole occurring after 30 min.

The quantitative significance of the figures quoted in Table 1 must be interpreted with caution for it is clear that the degree of enhancement observed is dependent not only on the rate of biological activation of the carcinogen but also on the concentration of carcinogen added. Thus, for 1 mM benz(a)pyrene maximal enhancement is observed within 5 min whereas for acetamidofluorene maximal enhancement is not achieved

Superficially, some correlation exists between the enhancement of biphenyl 2-hydroxylase activity and the degranulation of the endoplasmic reticulum but there are marked differences in other features of these two systems. For example, the carcinogen invoked biphenyl 2-hydroxylase enhancement is more rapidly mediated than degranulation; is considerably more susceptible to storage damage; and is not affected by EDTA (Table 1) which efficiently degranulates the rough endoplasmic reticulum11. The rapidity and relative lability of the carcinogen-stimulated increase of biphenyl 2-hydroxylase activity suggests that the carcinogens are modifying the membranes of the endoplasmic reticulum, perhaps invoking a conformational change which might subsequently lead to membrane damage

These results indicate that the study of the in vitro enhancement of biphenyl 2-hydroxylase in hepatic microsomes in the presence of a NADPH-regenerating system may prove a basis for a preliminary screening system which, in conjunction with other such tests, might be of considerable value in detecting potential carcinogens.

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<sup>\*</sup>All compounds were dissolved in 1.15% KCl and 1% Tween 80 and added to the incubation mixture to give a final concentration of  $5\times10^{-4}$  M except those in italics which were added as a solution in groundnut oil (Saladin) (0.5 mg in 0.5 ml) which also gives an approximate concentration of  $5\times10^{-4}$  M. Preincubation in each case was for 10 min.

<sup>†</sup>Indicates test results significantly different from control values

### Correlation of erythrocyte catechol-O-methyltransferase activity between siblings

CATECHOL-O-METHYLTRANSFERASE (COMT) is the enzyme that catalyses the conversion of noradrenaline, adrenaline, dopamine, and other catechol compounds to their O-methylated metabolites<sup>1</sup>. COMT is• widely distributed in many tissues including the red blood cell (RBC)<sup>1,2</sup> and individual differences in the activity of this enzyme in human beings may be involved in the pathogenesis of various psychiatric and neurological diseases<sup>3,4</sup>. Inhibitors of COMT have been used experimentally in the treatment of Parkinson's disease and other neurological diseases<sup>5</sup>. COMT activity is decreased in red blood cells obtained from depressed women<sup>3</sup> and is increased in erythrocytes obtained from children with Down's syndrome<sup>6</sup>.

Although there has been interest in the determination of erythrocyte COMT activity in blood from patients suffering from neurological and psychiatric disease, little is known about factors that regulate RBC COMT activity in unselected human populations. Here we describe a study of erythrocyte COMT activity in blood obtained from a large, unselected population of young men and women, the results of which demonstrate a significant correlation of COMT activity between siblings and suggest that there may be at least two human populations with regard to levels of activity of this catecholamine degradative enzyme activity in the erythrocytes.

Blood samples were obtained from 373 unselected adolescents aged 16-18 yr as part of a survey of lipoprotein values in students attending the Rochester, Minnesota schools. All but one of the individuals studied were white. Blood was obtained at school in the morning after an overnight fast.

Blood samples were withdrawn by venipuncture into plastic syringes and were transferred immediately to 2 ml heparinised vacuum tubes. COMT activity was determined in every sample on the day that the blood was obtained by use of a radiochemical enzymatic assay that is a modification of the method described by McCaman<sup>7</sup> (Fig. 1). 3,4-Dihydroxybenzoic acid was used as a substrate for COMT. One unit of enzyme activity represented the formation of 1 nmol of 4-hydroxy-3-methoxybenzoic acid (vanillic acid) per ml of packed red cells per hour of incubation. All results were evaluated by standard statistical methods.

Figure 1 shows the frequency distribution of erythrocyte COMT values in blood obtained from 373 unselected young men and women aged 16-18 yr. The mean COMT activity for the entire population was  $11.3\pm4.2$  (mean  $\pm$  s.d.); that of females was  $10.9\pm3.9$ ; and the mean RBC COMT activity of the male subjects was 11.8 ± 4.5. The distribution of COMT activity in this population seems to include at least two subgroups, one with a mean COMT activity of approximately 6-7 units and another with a higher mean enzyme activity of 12-14 units. This bimodality is present in the distribution of enzyme activities of the population as a whole and among the COMT activities determined in blood from the 189 female subjects and the 184 male subjects included in the study (Fig. 1). The same bimodality in the distribution of erythrocyte COMT values was found in a separate study of RBC COMT activity in 257 adolescents, 115 girls and 142 boys, age 13-15 yr in which COMT activity was determined in somewhat different assay conditions. In this younger population, as in the age 16-18 yr group, bimodality was present in the distribution of COMT activity of the entire population as well as in the separate distributions determined for male and female subjects.

A highly significant correlation (r=0.485) was found between erythrocyte COMT activity present in blood obtained from the 56 sibling-sibling pairs present in the population of young men and women aged 16-18 yr (P<0.001). The scatter diagram in Fig. 2 illustrates the sibling-sibling correlation of erythrocyte COMT values in this population. There was no difference in the degree of correlation between brother-brother, brother-sister and sister-sister pairings. When random pairs of single children with no siblings were generated by using tables of

random numbers, no significant correlation of erythrocyte COMT activity was found between members of non-sibling pairs. A highly significant correlation (r=0.59, P<0.001) was also present in blood obtained from the 37 sibling-sibling pairs included among the 257 adolescents aged 13-15 yr who were studied.

One of the difficulties in the determination of COMT activity in erythrocytes is the possibility that the results obtained might be due partially to the presence of endogenous inhibitors or activators of the enzyme. Both erythrocytes and plasma

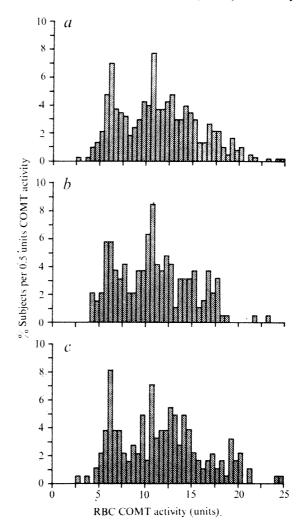
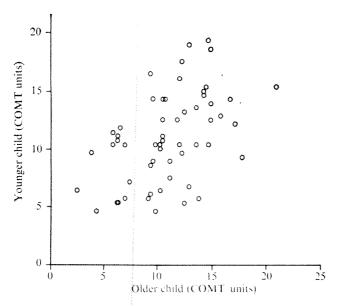


Fig. 1 Percentage frequency distribution of RBC COMT activity. The percentage of subjects with RBC COMT activity in successive 0.5 unit increments is shown for unselected populations that include: a, 373 males and females age 16-18 yr; b, 189 females and, c, 184 males considered separately. COMT activity was determined by a modification of the method of McCaman7. 200 µl of heparinised whole blood were added to 800 µl of ice-cold glass-distilled water containing 100 µl of a suspension of sodium Chelex-100. The lysed blood samples were mixed with the resin by rotation at 12 r.p.m. for 1 h at 4° C. After centrifugation at 700g for 10 min the supernatant was removed for the determination of COMT activity. 200 µl of the supernatant were placed in 15 ml stoppered conical centrifuge tubes to which 100 µl of a mixture of the following reagents final concentrations indicated) were added: Tris-HCl buffer, pH 7.5, 0.08 M; MgCl<sub>2</sub>, 10<sup>-3</sup> M; dithiothreitol, 4.2×10<sup>-3</sup> M; <sup>14</sup>C-methyl-S-adenosyl-1-methionine (specific activity 58 μCi μmol<sup>-1</sup>), 2.8×10<sup>-6</sup> M; non-radioactive S-adenosyl-1-methionine HCl, 20.2×10<sup>-6</sup> M; 3,4-dihydroxybenzoic acid, 10<sup>-3</sup> M. The reaction mixture was incubated for 45 min at 37° C, and the reaction was storyed with 100 u. of 1. N. HCl. The product was reaction was stopped with 100 µl of 1 N HCl. The product was extracted into 5 ml of toluene. After centrifugation, 3.5 ml of the organic phase were removed and added to liquid scintillation counting vials that contained 10 ml of toluene fluor. The radioactivity of the samples was determined by liquid scintillation counting. Blank samples included all reagents except 3,4-dihydroxybenzoic acid. Haematocrits were determined on all blood samples, and results were expressed as nmol of vanillic acid formed per ml of packed red blood cells per hour. (Details of the method will be published elsewhere 15.)



Sibling-sibling correlation of RBC COMT activity. RBC COMT activity determined in blood from the younger sibling in a pair is plotted against the activity of RBC COMT in blood of the older sibling. r=0.4846; P<0.001.

contain magnesium, an ion that is essential for COMT activity<sup>1</sup>. Variations in the concentrations of proteins that bind either endogenous or exogenously added magnesium might also influence enzyme activity. Blood contains S-adenosyl-1methionine<sup>4</sup>, a cosubstrate for the COMT reaction<sup>1</sup>. As the enzyme activity in this study was determined by the use of radioactively labelled methyl-14C-S-adenosyl-1-methionine, variable quantities of endogenous S-adenosyl-1-methionine in the blood might influence the apparent activity of the enzyme measured in a given individual. Finally, an enzymatic activity in blood that is capable of demethylating the product of the COMT reaction has been described<sup>8</sup>. Individual variations in this enzyme activity might also introduce artefacts into the determination of RBC COMT activity.

To help ensure that the individual differences in erythrocyte COMT activity reported here represent differences in the activity of the enzyme and not variations in the concentration of divalent ions, endogenous S-adenosyl-1-methionine, or other unknown inhibitors or activators of the enzyme, partially purified rat liver COMT was added to 201 consecutive blood samples in which RBC COMT activity was determined. Rat liver enzyme was purified by a modification of the method of Axelrod and Tomchick1 as described by Coyle and Henry9 with the addition of a final step in which the enzyme was subjected to gel filtration on a G-200 column. The specific activity of this preparation was 551 nmol product per mg protein per hour incubation. The average recovery of rat liver COMT added to blood samples was  $99\pm1.3\%$  (mean  $\pm$  s.e.m.), and there was no difference in the recovery of exogenously added enzyme from samples with low or high endogenous COMT activity. In addition, when lysed blood samples of high COMT activity were mixed with lysates of low activity, the resultant enzyme activity was the expected arithmetic mean. The distribution of COMT values described here cannot be explained by an increased inhibition or decreased activation of samples with low activity as compared with lysates of high COMT activity, nor is the reverse true of samples with high COMT activity.

The factors that influence the activity of red blood cell COMT in erythrocytes from unselected human subjects are not well understood. The results of our study show that a significant sibling-sibling correlation occurs in RBC COMT activity. Thus, familial factors seem to be important in the regulation of levels of this enzyme in the human erythrocyte. The relative

contributions of heredity and shared environment to these familial factors, and the possible mechanism of inheritance (single gene or polygenic) will have to be determined by family and twin studies. The results of this study also suggest the existence of at least two subgroups of subjects in an unselected population, one with low and one with higher RBC COMI activity.

These results raise the possibility of significant familia differences in enzymatic O-methylation of catecholamines it man. It has been reported previously that the activity of another catecholamine degradative enzyme, monoamine oxidase it human platelets, is subject to genetic control 10,11. The serum activity of the catecholamine biosynthetic enzyme dopamine-\$ hydroxylase (DBH), the enzyme that converts 3,4-dihydroxy phenylethylamine (dopamine) to noradrenaline, is affected by genetic factors. A striking sibling-sibling correlation exists for serum DBH activity12; this correlation is greater in blood samples obtained from monozygotic than in those obtained from dizygotic twin pairs13; and the results of recent studies are compatible with the existence of an autosomal recessive allele for very low serum DBH activity in man with a gene frequency of approximately 20% in the population studied14 The data that have been presented here with regard to a siblingsibling correlation of COMT activity may represent an addi tional example of the importance of familial factors in the regulation of the activities of catecholamine biosynthetic and degradative enzymes in man. Although RBC COMT is bio chemically similar to COMT in other tissues2, the identity o erythrocyte COMT with COMT in other human tissues ha not been established. Further studies will be necessary to deter mine whether the findings reported here bear a relationship to a possible role of catechol-O-methyltransferase in human disease states.

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### A reappraisal of human β MSH

A PEPTIDE containing 22 amino acids has been isolated from human pituitaries and, because of its structural resemblance to the octapeptide hormones previously isolated from pig, ox and sheep pituitaries and its similar pigment-dispersing biological activity in lower vertebrates, has been called  $\beta$ -melanocyte stimulating hormone ( $\beta$  MSH)<sup>1</sup>. The discovery and structural analysis of two related sheep pituitary peptides<sup>2.3</sup>  $\beta$ - and  $\gamma$ -lipotrophin ( $\beta$  LPH and  $\gamma$  LPH)<sup>1</sup> which showed that  $\gamma$  LPH comprised the first 1–58 amino acids of  $\beta$  LPH and,  $\beta$  MSH of sheep origin, the 41–58 sequence of both these molecules (Table 1), led Chretien and Li to suggest that  $\beta$  MSH was formed by enzymatic cleavage of  $\beta$  LPH,  $\gamma$  LPH representing the intermediate compound.

It has been shown that the human pituitary contains peptides identical to  $\beta$  lipotrophin and  $\gamma$  lipotrophin<sup>4,5</sup>, and it is evident that 'human  $\beta$  MSH', although it differs in length from the other mammalian  $\beta$  MSHs, is also derived by cleavages of these molecules. The very close correspondence of the human  $\beta$  MSH sequence to the 37–58 region of sheep  $\beta$  LPH (and what is known of the structure of human  $\beta$  LPH (ref. 4)) can be seen in Table 1.

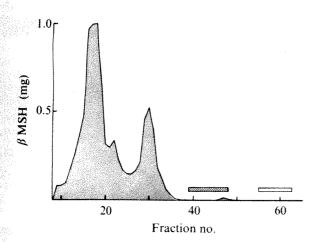


Fig. 1 Fractionation of a human pituitary gland extract on a BioGel P6 column eluted with 1 M acetic acid (column dimensions 90 cm  $\times$  1.5 cm). The shaded area represents  $\beta$  MSH immunoreactive material. The cross-hatched block indicates the elution position of ACTH and the open block that of synthetic human  $\beta$  MSH.

In comparing the  $\beta$  MSHs of the different species, it was apparent to us that as well as its larger size, human  $\beta$  MSH shows other peculiarities not shown by the other mammalian  $\beta$  MSHs. It is more difficult to extract<sup>6</sup> and is localised in the pars listalis rather than the pars intermedia<sup>7</sup>—a lobe which is rudimentary in man. These anomalies have persuaded us that he nature and role of the human  $\beta$  MSH molecule needs a more critical reappraisal. Here we describe our preliminary findings, which seem to throw considerable doubt on the actual existence

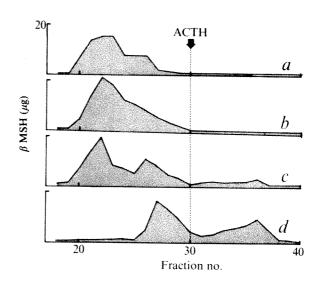


Fig. 2 Samples were removed at timed intervals from a 1 M acetic acid extract of human pituitaries, boiled to prevent further enzyme activity and chromatographed on a BioGel P6 column (90 cm  $\times$  1.5 cm). The distribution of  $\beta$  MSH-like immunoreactive material in each fraction is indicated by the shaded peaks. The vertical dotted line represents the approximate elution position of ACTH, and serves as a marker to show that prolonged extraction in 1 M acetic acid can lead to the formation of  $\beta$  MSH-like material of a smaller molecular size than ACTH.  $\alpha$ , Zero time;  $\beta$ , 0.5 h;  $\alpha$ , 2, h;  $\alpha$ , 16 h.

of human  $\beta$  MSH, and indicate that it may be an artefact of the  $\beta$  LPH molecule formed during certain extraction procedures.

A radioimmunoassay for human  $\beta$  MSH, employing a synthetic human  $\beta$  MSH preparation (CIBA/Giegy) for standardisation and iodination, was developed, and cross-reaction with ACTH and  $\alpha$  MSH was shown to be less than 0.1%. Human  $\beta$  and  $\gamma$  LPH were shown to be equipotent on a molar basis in this assay, and gave curves parallel to the standard.

Human pituitaries (frozen or acetone dried) were extracted in several different ways, assayed, and the extracts applied to Biogel P6 columns and eluted with acetic acid<sup>5</sup>. The pituitaries contained substantial amounts of  $\beta$  MSH immunoreactive material at concentrations corresponding to those reported by Abe and his colleagues<sup>8</sup>. After gel filtration, however, none of the  $\beta$  MSH immunoreactive material behaved as expected for human  $\beta$  MSH, but appeared as high molecular weight material eluting in fractions before ACTH, which has 39 amino acid residues (Fig. 1). Two peaks were identified and further purified by ion-exchange chromatography<sup>5</sup>. The largest molecular weight peak, which predominated in all extracts studied, had an amino acid composition closely resembling  $\beta$  LPH, and the other peptide resembled the NH<sub>2</sub>-terminal 58 amino acid sequence of human  $\beta$  LPH ( $\gamma$  LPH).

Further studies suggested that β MSH was only derived by enzymatic cleavage of these molecules during certain extraction procedures, most commonly those involving the use of dilute

1	17		į į	40	00
LPH (NH <sub>2</sub> ).	ALA-GLU-LYS-LYS-ASP-SER-GLY-PRO tot determinedLYS	-TYR-LYS-MET-GLU-HIS-	-PHE-ARG-TRP-GLY-SER-PRO-PRO	D-LYS-ASP-LYS-ARG	90 (COOH)
· n	ot determinedLYSGLU	ARG			
	,	* .	!		Huma
MSH	H-ALA-GLU-LYS-LYS-ASP-GLU-GLY-PRO	-TYR-ARG+MET-GLU-HIS-	PHE-ARG-TRP-GLY+SER-PRO-PRO	)-LYS-ASP-OH	Huma
	(I)	1			
	H-ASP-SER	LYS		(18)	Sheep
		*	•		

acid. For example, the B LPH in human pituitaries was readily degradable in extracts prepared using 6% acetic acid, unless enzyme activity in the tissue was destroyed by heating or lowering the pH (Fig. 2). A proportion of the degraded fragments resembled human \( \beta \) MSH in their physicochemical properties.

Our interpretation of how B MSH was found in human pituitaries is exceptionally well supported in the literature. It is evident, for example, that the original isolation of human β MSH was made from a side-fraction of a growth hormone extraction procedure1, and it seems quite probable, as a result of the employment of mild extraction conditions to obtain the growth hormone, that degradation of  $\beta$  LPH and  $\gamma$  LPH took place during this or subsequent procedures to form  $\beta$  MSH. Lerner and his colleagues6 have reported several times in the past decade how, if direct extraction of pituitaries is carried out in strong acid conditions (conditions which do not favour proteolysis), human β MSH cannot be isolated.

We have recently been extending our studies to immunoreactive B MSH in human plasmas, samples of which have now been taken from patients with Nelson's syndrome and a normal subject following pyrogen administration. In all cases, the concentrations of \$\beta\$ MSH immunoreactive material were very high, but on gel filtration none of this material eluted in the expected position of human \( \beta \) MSH (Fig. 3). The bulk of immunoactivity resembled β LPH and/or γ LPH in its elution properties, but some was always found in the void volume (a molecular weight of greater than 20,000 was indicated). This latter material may be either an even larger precursor molecule, (such as 'Big ACTH'9), or it may be β LPH non-covalently linked to a plasma protein.

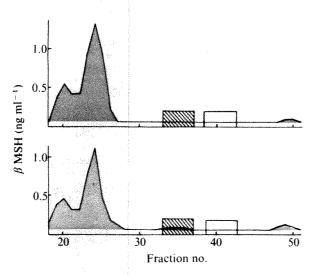


Fig. 3 Fractionation of plasma from two patients with Nelson's syndrome on a BioGel P6/P10 column eluted with frog-Ringer/ albumin buffer. (Column dimensions: P6, 100 cm  $\times$  1 cm, P10, 33 cm  $\times$  1 cm.) The shaded peaks represent  $\beta$  MSH immunoreactive material. The cross-hatched block indicates the elution position of ACTH and the open block that of synthetic human β MSH. Most of the β MSH immunoreactivity eluted in a peak corresponding in molecular size to \( \beta \) LPH.

The stability of β MSH immunoactivity in fresh plasma, thawed plasma or whole blood bears a much closer similarity to the behaviour of β LPH and γ LPH than to synthetic human  $\beta$  MSH, when they are exogenously added to the fluids. The larger peptides are considerably more stable than the B MSH.

In conclusion, our findings suggest that it is likely that radioimmunoassays for human \( \beta \) MSH cannot actually be measuring this hormone, as it does not normally exist. It seems instead that human β MSH radioimmunoassays will be measuring other peptides, the most important of which in pituitary tissue is  $\beta$  lipotrophin, and in plasma, either  $\beta$  LPH or  $\gamma$  LPH. This conclusion readily explains why human B MSH differs so much from the other mammalian  $\beta$  MSHs. It is especially evident that the well-documented association between B MSH and ACTH in man<sup>11</sup> (the two peptides appear to be synthesised and stored in the same pituitary cell and released together in the same physiological or pathological situations) is really an association between \$ LPH and ACTH.

A peptide resembling a true β MSH has been found in extracts of several tumours associated with the ectopic ACTH syndrome<sup>12,13</sup>. On gel filtration, this peptide elutes in the same position as a bovine \( \beta \) MSH standard, but not in the position of the synthetic human  $\beta$  MSH standard. This finding is not unexpected, as it ties in very well with our findings on the other melanophore stimulating hormone, a-MSH, and the 'corticotrophin-like intermediate lobe peptide' (CLIP), both of which are derived by cleavage of ACTH in pituitary pars intermedia cells14, and are also found in tumours associated with the ectopic ACTH syndrome in man15.

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### Possible pharmacological and theoretical implications of X-ray structure of the tricyclic antidepressant imipramine

IMIPRAMINE was first shown in 1958 to be effective in the treatment of endogenous depression. It is one of the most widely prescribed members of the tricyclic antidepressant group of compounds which have been chosen for the treatment of the average depressed patient2. It is perhaps surprising that not a single member of this important group of psychotropic drugs has been investigated by X-ray analysis. There is now considerable evidence that a functional abnormality of one or more of the biogenic amine systems of the brain is involved in the aetiology of the depressive or affective disorders. Amines which have been investigated most thoroughly in this respect are noradrenaline and 5-hydroxytryptamine (5-HT). The synaptic action of these putative

$$t_1$$
 $t_2$ 
 $t_3$ 
 $t_4$ 
 $t_5$ 
 $t_7$ 
 $t_7$ 

Fig. 1 Molecular diagrams of imipramine hydrochloride as determined by X-ray analysis. a, Molecule A; b, molecule B. An asterisk marks the atoms used in torsion angle calculations where an ambiguity in definition occurs. Torsion angle values for A and B are as follows (A is quoted first):  $\tau_1 = 137^{\circ}$ ,  $59^{\circ}$ ;  $\tau_2 = 180^{\circ}$ ,  $161^{\circ}$ ;  $\tau_3 = 174^{\circ}$ ,  $61^{\circ}$ ;  $\tau_4 = 169^{\circ}$ ,  $-172^{\circ}$ .

neurotransmitters is thought to be terminated largely by an active uptake back into the presynaptic nerve ending, and the rat brain is known to possess separate neuronal uptake systems for noradrenaline and 5-HT7. The most striking neurochemical effect of tricyclic antidepressants is their potency in inhibiting these transport processes', and indeed it has been suggested that this effect may be responsible for their clinical ameliorative action. Structure-activity relationships for various imipramine analogues as inhibitors of noradrenaline<sup>8</sup> and 5-HT<sup>10,11</sup> uptake have been reported and recently a possible molecular mechanism for this inhibitory effect has been proposed12. In an attempt, therefore, to gain some insight into the conformational requirements for a compound to be a potent inhibitor of noradrenaline and 5-HT uptake, we are investigating the X-ray structures of a number of tricyclic antidepressants<sup>13</sup>; that of imipramine is reported here.

Well-formed crystals of imipramine hydrochloride, ranging from 0.1-1.5 mm in size in the largest dimension, were grown from a chloroform-xylene mixture. The space-group was uniquely determined as P2<sub>1</sub>/c with eight molecules in the monoclinic cell of dimensions a=11.303 Å, b=29.277 Å, c=14.282 Å,  $\beta=130.91^{\circ}$ , thus requiring two distinct molecules in the crystallographic asymmetric unit. This gives rise to the interesting possibility of two conformations in the solid state structure. Diffractometer data were collected and normalised structure factors (Es) derived. The structure was solved using reflections with  $E \ge 1.2$  by a centrosymmetric multi-solution direct methods program (G. M. Sheldrick, private communication). At present, the conventional R factor is 0.057; complete details of the fully refined structure will be published elsewhere. Figure 1 illustrates the close similarity of the geometry of the tricyclic ring system for the two molecules of the asymmetric unit. The dihedral angle between the planes of the benzene rings is 130° and 123° for molecules A and B, respectively, while the angles between each benzene ring and the plane N-C(methylene)—C(methylene) of the seven-membered ring are 18° and 116° (for A) and 2° and 125° (for B), the latter set of values reflecting the twist in the ring system. The ring nitrogen atom is pyramidal, lying 0.19 Å (for A) and 0.29 Å (for B) from the plane formed by the three carbon atoms to which it is bonded.

The dimethylaminopropyl chains show a definite variation in conformation. The values approximate to a staggered conformation with respect to the N-C(benzene) bonds. From  $\tau_1$  onward, A is almost fully extended, that is, all torsion angles are close to  $\pm$  180°, while B folds back to  $\tau_3$ . In each case the amino nitrogen is hydrogen bonded to a Cl ion 3 Å distant. As the tricyclics are structurally dissimilar to noradrenaline and 5-HT, it is interesting to know what structural and conformational properties enable them to be such potent inhibitors of the neuronal transport of these two putative CNS transmitters. Kinetic studies indicate that they are competitive inhibitors of these uptake systems in the peripheral14 and central nervous systems9 and in blood platelets18, and are, therefore, probably binding to the same site as the endogenous substrates. The most obvious function for the terminal amino group of imipramine is presumably to block the binding site with which the primary amine function of noradrenaline or 5-HT normally interacts12. It is possible that one of the benzene rings of the tricyclic nucleus blocks the binding site usually occupied by the aromatic ring of noradrenaline<sup>12</sup>. Similarly, a larger portion of the tricyclic system may also effectively block the binding site of the indole nucleus of 5-HT. Previous studies18-18 have shown that the most likely conformation of phenylethylamine analogues and catecholamines at the uptake site is very similar to the fully extended trans form which has been shown to be the preferred conformation of noradrenaline in the solid state<sup>19</sup>, in solution<sup>20</sup> and in vacuo by theoretical calculations<sup>21</sup>. A comparison of the distances

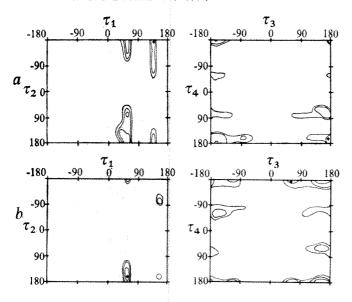


Fig. 2 Potential energy maps for imipramine. a, Molecule A; b, molecule B. Energies were calculated by atom-atom summation of van der Waals interactions as programmed by Motherwell<sup>23</sup>. Contours are at 1 kcalorie mol<sup>-1</sup> intervals and areas not contoured are regions of high energy corresponding to close interatomic approaches and atomic collisions. Heavy crosses indicate the conformations found in the crystal structure.

of the terminal nitrogen atom from the centres of the aromatic rings in both conformations of imipramine with that in noradrenaline19 shows that in the case of conformation B the above proposals12 seem reasonable, although exact coincidence is not attained and may not in fact be necessary. The possibility of conformations other than those found in the crystal, existing in solution can not be excluded (as a recent NMR study of imipramine and its hydrochloride has shown<sup>22</sup>) and a potential energy approach toward this problem has been applied.

Figure 2 shows potential energy maps obtained by varying, in each case, two torsional angles of the molecular side chain whilst the remaining two are fixed, in this particular case, at values observed in the X-ray analysis, to reduce the problem to one in two dimensions. This limitation is not a prerequisite, however, and the same approach can be used iteratively, without any previous assumption of  $\tau$ values, to study the complex arrangement of more than two variables. It is apparent from Fig. 2 that other conformations of similar energy to that of the crystal form could exist, as indicated by a number of minima in the maps other than those corresponding to the crystal position. There seems to be no a priori method of distinguishing which conformation is most likely in the absence of packing constraints (which are important in the crystalline form) and further studies with more rigid tricyclic antidepressants, which are currently underway in our group, may throw light on this problem.

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### Transcriptional control of α-foetoprotein synthesis in developing mouse liver

α-Foetoprotein (αFP) is a dominant serum protein in many mammalian species during embryonic and early postnatal life It is hardly detectable in the adult body but is often found in an elevated quantity in the serum of individuals with hepatocarcinomas and teratocarcinomas1-5

αFP is synthesised in the yolk sac and foetal liver but it is not known whether the decrease in aFP synthesis in adult liver is due to an alteration in mRNA synthesis or in mRNA translation. To answer this question we have extracted RNAs from polysomes of foetal and adult mouse liver and measured their abilities to direct the synthesis of aFP in cell-free systems. Under the conditions used, the translation of polysomal RNA depends on the presence of high-salt extracts of ribosomes (ribosomal wash) which are known to contain factors involved in the initiation of polypeptide synthesis. It has been possible to determine whether the reason for the reduction in aFP synthesis in adult liver resides in the mRNA fraction or in the ribosomal wash.

Total polysomes were prepared from the liver of adult ICR mice of 14 day foetal mice according to Falvey and Staeheline. RNA was extracted from the polysomes using 1% sodium dodecyl sulphate (SDS) and a mixture of phenol and chloroform?. In some experiments, polysomal RNA was further fractionated by treating the total polysomes with 1% SDS, 5 mM EDTA and 20 mM NaCl followed by sucrose density-gradient centrifugation<sup>8</sup>. RNA sedimenting at 15-20S

Table 1 Synthesis of αFP with ribosomes and high speed supernatant from foetal and adult liver

Ribosome	High speed	Foetal polysomal	αFP
	supernatant	RNA	(c.p.m.)
Foetal	Foetal	+	387
Foetal	Adult	+	411
Adult	Adult		396
Foetal or adult	Foetal or adult	territorial -	93

The components for protein synthesis were described in the legend to Fig. 1. The samples were incubated at 30° C for 60 min and centrifuged at 149,000g for 2 h. Immunoprecipitation was carried out using 500 μl samples as described previously with the following modifications. The reaction was performed in the presence of 1% Triton X-100 and 1% sodium deoxycholate (DOC) to minimise non-specific precipitation 16-18. The samples were then centrifuged through a 0.9 M sucrose cushion containing 1% Triton X-100, 1% DOC and 10 mM each of leucine, valine and phenylalanine. The pellets were dissolved in 1% sodium dodecyl sulphate-4 M urea-1% β-mercaptoethanol at 45° C for 30-60 min and the radioactivity was measured with 10 ml Aquasol (NEN Canada, Montreal, Quebec).

Figures are averages of three assays.

was recovered by precipitation with ethanol and used as 18S RNA. This RNA fraction was enriched with αFP mRNA (K. K., D. W. O., T. I., and T. T., unpublished) as well as albumin mRNA<sup>9-11</sup>. Ribosomal wash was prepared according to Prichard *et al.*<sup>12</sup>. The cell-free systems from the adult mouse liver (adult liver cell-free system) and 14 day foetal liver (foetal liver cell-free system) were prepared as described previously<sup>11</sup>.

Figure 1 shows polyacrylamide gel electrophoretic analysis of proteins produced in the foetal liver cell-free system in the presence of foetal polysomal RNA and foetal ribosomal wash. Four main radioactive fractions were resolved, two of which were comigrating with carrier αFP and albumin, respectively. Additional evidence that they were in fact newly formed αFP and albumin was obtained by immunoprecipitation followed by gel electrophoresis<sup>13,14</sup>. Control samples incubated without polysomal RNA or ribosomal wash showed little radioactivity throughout the gel.

The requirements for  $\alpha FP$  synthesis in the cell-free system were studied by substituting foetal liver components with corresponding preparations from adult liver. In this experiment,  $\alpha FP$  synthesis was assayed by measuring radioactivity in the precipitate formed with antiserum against mouse  $\alpha FP$ . Specificity of the reaction was examined using various control samples and will be published elsewhere. Table 1 shows that substitutions of foetal ribosomes and high speed supernatant fraction with adult counterparts did not affect the synthesis

Table 2 Synthesis of αFP and albumin in the adult liver cell-free system

Exp.	RNA	Ribosomal wash	αFP (c.p.m.)	Albumin (c.p.m.)	αFP/Albumin
I	Foetal	Foetal	335	130	2.6
	Foetal	Adult	201	76	2.6
	Adult	Foetal	54	167	0.3
	Adult	Adult	25	120	0.2
II	Foetal	Foetal	288	126	2.3
	Foetal	Adult	248	83	3.0
	Adult	Foetal	79	147	0.5
	Adult	Adult	66	105	0.6

Conditions for protein synthesis were the same as described in the legend to Fig. 1, except that 'run-off' 80S ribosomes and high speed supernatant were prepared from adult mouse' liver. The adult ribosomal wash was used at 1.5 mg protein ml<sup>-1</sup>. In I and II, the '18S' RNA from foetal and adult polysomal RNA was used at 3.5 A<sub>260</sub> ml<sup>-1</sup>. The samples were incubated at 30° C for 60 min and centrifuged at 149,000g for 2h. The supernatant was removed and divided into two aliquots. One (500 μl) was analysed for αFP as described in the legend to Table 1. The other was analysed for albumin in a similar manner using rabbit antiserum against albumin. Appropriate background counts, obtained in the absence of RNA (95-190 c.p.m.), were subtracted.

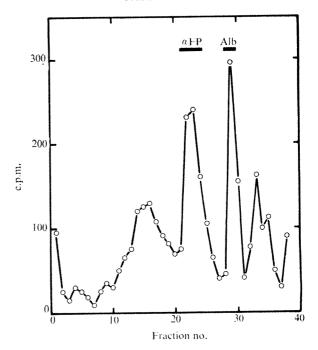


Fig. 1 Polyacrylamide gel electrophoretic analysis of proteins produced in the presence of foetal polysomal RNA in the foetal liver cell-free system. The reaction mixture (3 ml) contained the following (per ml): 2 A<sub>280</sub> foetal 'run-off' 80S ribosomes, 6 mg foetal high-speed supernatant protein, 1 μmol ATP, 0.3 μmol GTP, 10 μmol phosphocreatine, 50 μg mouse liver tRNA, 35 μCi <sup>3</sup>H-leucine (1 Ci mmol<sup>-1</sup>), 10 μCi <sup>3</sup>H-valine (2 Ci mmol<sup>-1</sup>), 10 μCi <sup>3</sup>H-phenylalanine (6.15 Ci mmol<sup>-1</sup>), 25 nmol each of 17 other amino acids, 20 mM Tris-HCl, pH 7.8, 3.5 mM MgCl<sub>2</sub>, 70 mM KCl, 6 mM β-mercaptoethanol, 0.5 mM dithioerythritol, 750 μg foetal ribosomal wash protein and 3.5 A<sub>280</sub> foetal polysomal RNA. The sample was incubated at 30° C for 60 min and centrifuged at 149,000g for 2 h, the supernatant adjusted to pH 4.8 and the precipitate removed. (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was added to the supernatant to 40% saturation at 0° C and the precipitate was dissolved in 20 mM Tris-HCl (pH 7.8)–150 mM NaCl. This resulted in about fourfold purification of αFP and albumin<sup>11</sup>. Electrophoresis was performed with neuraminidase-treated αFP and albumin as carriers<sup>14.19</sup> and radioactivity was measured as described previously<sup>13,14</sup>. The bars indicate positions of carrier αFP and albumin (Alb).

of  $\alpha FP$ . This suggested that either mRNA or ribosomal wash was responsible for the regulation of  $\alpha FP$  synthesis.

The synthesis of  $\alpha FP$  in the presence of foetal or adult polysomal RNA in combination with foetal or adult ribosomal wash, was therefore examined, using an assay of the synthesis of albumin as an internal control. The results showed that when foetal polysomal RNA was used as a template,  $\alpha FP$  synthesis exceeded albumin synthesis two-to threefold with either foetal or adult ribosomal wash (Table 2). When adult polysomal RNA was used as a template, however, the synthesis of  $\alpha FP$  decreased drastically with either foetal or adult ribosomal wash. Albumin synthesis was relatively unchanged,

Table 3 Synthesis of αFP and albumin in the mouse sarcoma cell-free system

 RNA	Ribosomal wash	αFP (c.p.m.)	Albumin (c.p.m.)	αFP/Albumin	
Foetal	Foetal	308	133	2.3	
Foetal	Adult	365	145	2.5	
Adult	Foetal	263	322	0.8	
Adult	Adult	99	164	0.6	

Conditions for protein synthesis were the same as that described in the legend to Fig. 1, except that 200 μl S-30 of mouse sarcoma 180 replaced 'run-off' 80S ribosomes and high speed supernatant. Both foetal and adult RNA preparations used were total polysomal RNA (3.5  $A_{260}$  ml<sup>-1</sup>). The samples were incubated at 30° C for 2 h and 330 μl post-ribosomal supernatant was used for the assay of αFP or albumin as described in the legend to Table 1. Appropriate background counts, obtained in the absence of RNA (106-225 c.p.m.), were subtracted.

Table 4 Synthesis of αFP and albumin in the presence of nuclear and cytoplasmic RNA from adult mouse liver

RNA	αFP (c.p.m.)	Albumin (c.p.m.)	αFP/Albumin
Nuclear	7	21	0.3
Cytoplasmic	18	184	0.1
Foetal polysomal	217	57	3.8

Assay conditions were the same as described in the legend to Table 3. RNA was used  $3\,A_{250}\,\mathrm{ml}^{-1}$ . Appropriate background counts, obtained in the absence of RNA (105 c.p.m. for  $\alpha$ FP, 156 c.p.m. for albumin), were subtracted.

and as a result, the ratio of aFP synthesis to albumin synthesis decreased to 0.2-0.6. This indicated that aFP mRNA was deficient in the adult polysomal RNA although the factors necessary for its translation were present in adult liver. The counts reported here were relatively low but a series of experiments showed that the results were highly reproducible as shown by two examples in Table 2. Higher counts of aFP have since been obtained by including 3H-glutamic acid in the reaction mixture. This is presumably due to a high content of glutamic acid in aFP (E. F. Zimmerman, unpublished). These results confirm the conclusion stated above.

A similar experiment was conducted in a heterologous cell-free system using an S-30 fraction prepared from mouse sarcoma 18015. Translation of polysomal RNA in this system was also dependent upon the addition of ribosomal wash (K. K., D. W. O., T. I., and T. T., unpublished). Table 3 shows that the synthesis of aFP depended on the source of mRNA but not of ribosomal wash, in agreement with the results obtained with the liver cell-free system.

Although the above experiments indicated the deficiency of active aFP mRNA in the adult liver polysomes the possibility existed that it might be present in the nucleus or in the cytoplasm in a free form. This was tested by fractionating the adult liver into nuclei and cytoplasm, extracting RNA from each fraction and measuring its ability to synthesise aFP and albumin. Table 4 shows that the synthesis of aFP by the nuclear or cytoplasmic RNA was small, suggesting that the level of aFP mRNA in adult liver is in fact greatly reduced.

Another possibility, that aFP mRNA may have been degraded during extraction from adult liver, can be ruled out as albumin mRNA was extracted in an active form from the same source.

We conclude that the synthesis of  $\alpha FP$  in the developing mouse liver is regulated at the level of mRNA. Several steps are involved in the production of mRNA and its appearance in the cytoplasm, for example, transcription, transportation, maturation and the addition of poly(A). Further study is necessary to determine which one of these reactions is responsible for the reduced synthesis of aFP mRNA in adult liver. It is conceivable that such control is relaxed in hepatomas and teratomas, leading to an elevated level of aFP in the serum. Elucidation of the regulatory mechanism involved may thus throw light on the process of malignant transformation as well as normal development.

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### In vitro neoplastic transformation of epithelial cells of rat urinary bladder by nitrosamines

ORAL administration of N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) selectively induces tumours in the urinary bladder of rats and mice1-3. This organ specificity can be explained to some extent by the metabolic fate of the compound and the carcinogenic effect of its major urinary metabolite on the bladder; Okada and Suzuki4 found that BBN given to a rat was soon excreted as its carboxy derivative, N-butyl-N-(3carboxypropyl)nitrosamine (BCPN). Subsequent tests for carcinogenicity of BCPN showed that it too caused bladder tumour in rats5,6. Furthermore, BBN can be converted to BCPN when incubated with bladder mucosa or liver tissue? These findings suggest that BCPN is responsible for carcinogenesis of the bladder by BBN, or that both BCPN and BBN are directly carcinogenic to bladder epithelium. We have therefore studied the in vitro effect of both BCPN and BBN on

Table 1 Culture of epithelial cells of urinary bladder in various

Experiment series	Age of donors	No. c	of flasks tested	s contair flasks v	ning cell vith medi	strains/ ium* of	No. of
0.1	(weeks)	A	В	C	D	E	F
0†	12, 15	0/6		0/4		0/4	
1	12	0/1	0/1	0/1	2/2	0/1	2/2
3‡	8	0/1	0/1	0/1	2/2	0/1	1/1
•		•			(0/1)		(0/1)
6	9	0/1	0/1	0/1	1/2		
8‡	_39	0/1	1/2	0/1	3/3	0/1	2/2
•	•	•	*	•	(1/1)		(1/1)
•	Total	0/10	1/5	0/8	8/9	0/7	5/5

A, Plain medium; B, plain medium plus urea; C, plain medium plus BCPN; D, plain medium plus urea and BCPN; E, plain medium plus BBN; F, plain medium plus urea and BBN.

\*Concentration of BCPN or BBN was 0.03%.

Accumulated results of preliminary experiments

Data in parenthesis shown in series 3 and 8 indicate the results of culture in 0.015 and 0.06% nitrosamine-containing medium, respectively.

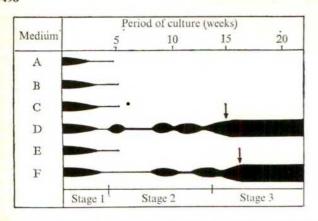


Fig. 1 Growth patterns of epithelial cells of the bladder in various media. Rats were killed by bleeding from the aorta. After terminal ligature of the bladder, about 1 ml of 0.25% trypsin solution containing 0.05% disodium salt of ethylenediaminetetraacetic acid was injected into the bladder lumen. The inflated bladder was separated from the animal and incu-The inflated bladder was separated from the animal and incu-bated in a humidised CO<sub>2</sub> incubator for 45 min at 37° C, and its contents were collected by cutting one end. The bladder was turned inside out and the mucosa was squeezed gently with forceps and washed with 10 ml of Eagle's minimal essential medium (MEM). A combined cell suspension of the contents and the washings of five bladders was transferred to centrifuge tubes, and placed in an ice bath for 3 min to remove sedimented debris and nematode worms. The cells were collected by centrifugation, washed once with MEM and suspended in MEM supplemented with 10% foetal calf serum and 60 μg kanamycin ml<sup>-1</sup> (plain culture medium). Cell suspension containing 300 × 103 cells in 5 ml was inoculated into a square type tissue culture flask (MA 30) which was sealed with a rubber stopper. After culture for 3–4 d at 36° C the medium was replaced by medium A, B, C, D, E or F (described in the legend to Table 1). Nitrosamines were dissolved in physiological saline making 0.3% stock solutions, and urea was dissolved in MEM making 0.5% stock solutions, and urea was dissolved in MEM making 0.5%. solution just before preparation of culture media. Concentrations of nitrosamine and urea in culture media were adjusted to 0.03 and 0.05%, respectively. Each medium was replaced every 7-10 d with the medium of the same constitution as the first. The black area in the figure indicates the apparent growth patterns of epithelial cells in experimental series 3 (see Table 1). The arrow indicates the time of passage.

epithelial cells of rat bladder. In preliminary experiments we had failed to induce growth of these cells, but we found that addition of urea to medium containing a nitrosamine induced growth. We have now established epithelial cell strains from normal cell by culturing them in the presence of BCPN plus urea or BBN plus urea and found that they grow as cancers in syngeneic animals.

Cells were obtained by trypsinisation from bladder mucosa of adult male rats of the inbred ACI/N strain (usually five of the same age for each series of experiments). Cells were cultured in Eagle's minimum essential medium supplemented with 10% foetal calf serum and 60 µg kanamycin ml<sup>-1</sup> (referred to here as plain culture medium) for 3–4 d until they had fixed and spread on the surface of a tissue culture flask. Then the cells were cultured in the presence of urea or BCPN or BBN or a nitrosamine plus urea without removing these compounds from the medium until the cell strain was established. In plain culture medium epithelial cells became polynuclear

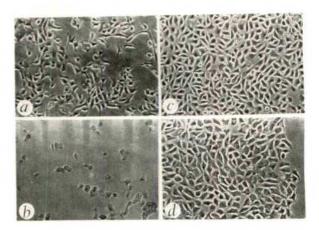


Fig. 2 Culture of cells in medium containing nitrosamine and urea. a, Initial cell growth in medium containing BCPN and urea at 5 weeks; b, degeneration of the cells at 6 weeks; c, cell strains established by BCPN and urea (BES3P) and (d) by BBN and urea (BES3B). (Phase contrast,  $\times$ 100.)

and died after 4-5 weeks (Fig. 1). In medium containing urea alone or a nitrosamine alone the fate of the cells was similar. In medium containing urea and BCPN or urea and BBN some cells survived for 4-8 weeks, although many died during this period (stage 1). The surviving cells started to divide after this latent period (Fig. 2a) and formed one or two colonies per flask. When a focal monolayer formed, many cells died in 1-2 weeks (Fig. 2b) (this is referred to here as contact death), but a few cells survived and divided again. This process was repeated two or three times (stage 2), and finally cells grew without contact death to form a confluent monolayer (stage 3). Usually it took 13-15 weeks for cells to reach the final stage. Confluent cells were trypsinised and passed to other flasks, thus establishing cell strains amenable to serial passage. Attempts to passage stage 2 cells, by trypsinisation or by scraping with a rubber policeman, resulted in rapid degeneration of the cells in a new flask. If medium containing both

Table 2 Backtransplantation of cells of established strains into syngeneic rats										
Cell strain and	culture	X-irradiation to recipient	No. of cells injected (×10°)	Injection site	No. of rats bearing tumour	Days until tumour examination				
BES3B-6	24	-	0.1	i.p.	2/2	183, 183* 100(2)				
	Cell strain and passage no.	Cell strain and culture passage no. (weeks) BES3B-6 24	Cell strain and culture recipient passage no. (weeks) BES3B-6 24 -	Cell strain and culture passage no. (weeks) BES3B-6 24 - No. of cells injected (×10°) 0.1	Cell strain and culture recipient injected site (×10°) BES3B-6 24 - 0.1 Injection site (×10°)  No. of cells Injection injected (×10°)  (×10°)  i.p.	Cell strain and culture recipient passage no. (weeks) BES3B-6  Period of X-irradiation to culture recipient injected (×10°)  Vo. of cells Injection site bearing tumour (×10°)  0.1  i.p. 2/2				

BES3B-6	24	-	0.1	i.p.	2/2	183, 183*
BES3B-7	25	_	1	i.b.	2/2	100(2)
BES3B-8	31	+	1	i.p.	1/2	64
BES3B-8	31	+	1	s.c.	2/2	64, 86
BES3P-4	25	_	2	i.b.	2/2	147, 212
BES3P-8	42	_	2	S.C.	3/3	128(3)
BES3P-8	42	+	2	s.c.	1/1	128
BES8B-6	31	+	5	i.p.	2/2	122, 128*
BES8B-6	31	+	5	s.c.	1/2	125
BES8P-5	31	• +	5	i.p.	2/2	125, 128
BES8P-5	31	. + .	5	s.c.	0/2	100/2) 129/2)
BES8U-2	27	-	• 2	S.C.	4/4	100(2), 128(2)

Cells, which had been kept by serial passages and by continuous culture in the medium containing nitrosamine and urea or urea alone, were cultured in several large flasks containing plain culture medium until they formed a confluent monolayer. Cells obtained by trypsinisation were suspended in Eagle's medium and counted. A known number of cells was injected into 8–9-week-old male ACI/N rats, some of them already irradiated with 300 r. The recipients were killed when tumours reached appropriate sizes for examination. Animals which did not develop tumours were observed until 200 d after transplantation. Cell strains are named as follows: BE means they derived from bladder epithelial cells; S number denotes the experimental series number, and the last character shows the drug added in the medium, that P, BCPN plus urea B, BBN plus urea; U, urea alone. i.p., intraperitoneal; s.c., subcutaneous; i.b., into bladder wall.

urea and nitrosamine was changed to plain culture medium at this stage, all cells died in several weeks without further growth, whereas cells in stage 3 or cells of the established strains could grow even in plain culture medium. Large fibroblast-like cells, which were always present with epithelial cells in the early stage of culture, grew to some extent, but always degenerated gradually and after 10 weeks few were detectable. Thus established cell strains consisted of pure epithelial cells (Fig. 2c and d).

Table 1 summarises the results of a similar experiment. In each series of experiments the fate of cells cultured in the media corresponding to those described above was similar to that shown in Fig. 1. Only cells from 39-week-old rats (series 8), however, continued to grow in medium containing urea alone. In this case the start of growth, 10 weeks after inoculation, was delayed relative to that of cells cultured in medium containing both urea and a nitrosamine but a confluent monolayer gradually formed. Cells of all established strains listed in Table 2 had epithelial shape and orientation, and most of them had more than two relatively large nucleoli, compared with normal epithelial bladder cells which have one or two such nucleoli. Modal chromosome numbers of the cell strains varied from 70 to 80 except BES3P strain whose modal number was 42. The tendency of cells to pile up in a monolayer was rarely demonstrated.

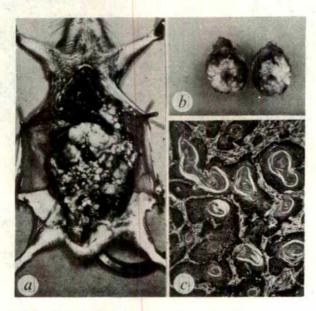


Fig. 3 Tumours developed from BES3B cells by intraperitoneal injection (a) and by intrabladder-wall injection (b), and histology of the tumour (c). (Haematoxylin and eosin,  $\times$  50.)

Tumorigenicity of cells of the established strains was tested by injecting them into adult ACI/N male rats, some of which had been X irradiated on the previous day (Table 2). All cell strains induced in medium containing both urea and a nitrosamine and one strain in medium containing urea alone gave rise to tumours, after injection subcutaneously, intraperitoneally or into the wall of the bladder. In 1-3 months the tumours grew to a palpable size. All tumours contained areas of squamous metaplasia, histologically designated squamous cell carcinoma. Tumour cells were arranged in a cystic or papillary form, containing a keratin pearl (Fig. 3c). Intraperitoneal BES3B, BES8B or BES8P cells gave rise to many tumour nodules in the peritoneal cavity of recipients (Fig. 3a), some causing death. Tumours produced by injection of cells were serially transplantable into untreated syngenic rats.

Our results show that epithelial cells of the urinary bladder of adult rats transform into neoplastic cells when treated with urea combined with either BCPN or BBN. Because fibroblast-like cells did not grow, nitrosamine plus urea must

affect epithelial cells specifically. This recalls the in vivo carcinogenesis of the bladder when BBN or BCPN induced very few tumours originating from cells other than epithelial cells \$1.5. Our experimental system is unique in the following ways. (1) The culture conditions reflect the in vivo condition for induction of bladder tumour by BBN-the concentration of nitrosamine in the medium is comparable with that of BCPN excreted in the urine when a carcinogenic concentration of BBN is administered in the drinking water (our unpublished data). Target cells are exposed continuously to nitrosamine as with in vivo carcinogenesis and urea is present in the medium. (2) Cell donors are adult animals and target cells come from the primary cultures, and not foetal or newborn animals as in previous experiments8. (3) Cells of the established strains develop cancers in untreated adult animals.

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### Effect of split doses of X rays on neoplastic transformation of single cells

Using cloned hamster embryo cells in culture, we have found that if an X-ray dose is divided into two fractions, more neoplastic transformations occur than if the same dose is given in a single exposure. This could have important implications for radiation protection, where in general radiation is accumulated in a number of small fractions rather than in a

single large exposure.

We used the in vitro system1.2, in which primary or secondary cultures of hamster embryo cells are seeded at low density to allow for clone formation. More cells are seeded as the dose increases to account for cell killing. Twenty-four hours elapse between plating and irradiation, in order for cells to attach, after which cultures are incubated in a humidified atmosphere of 5% CO<sub>2</sub> in air for 9-10 d. They are then fixed, stained and examined for the presence of transformed clones, which can be distinguished morphologically from controls. The transformed cells show ·a loss of contact inhibition3, and pile up randomly, in contrast to normal cells1,2,4-6 (Fig. 1). We have described the relationship between the morphology of the transformed cells and their tumorigenicity in vivo1.2; when injected into 2-3-week-old hamsters they give rise to fibrosarcomas (Fig. 2). Their ability to grow in semi-solid medium and in suspension, and their agglutinability by plant lectins have served as further criteria for transformation2.

Table 1	Neoplastic transformation in vitro following single and split doses of X rays
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		Control					Irra	diated		
Exp.	No. clones counted	Plating efficiency	Feeder present	No. transformed clones	Dose	No. clones counted	Feeder present	No. transformed clones	Transformed	Surviving
1	6,000	3.2	+	0	$75 (37.5 \times 2)$	2,081 3,002	+	14 32	0.675 1.065	0.89
2	5,080	2.5	+	0	75 (37.5×2)	9,800 8,430	+	70 95	0.715 1.129	0.90
3	3,002	2.0	+	0	75 (37.5×2)	2,120 2,020	+	10 17	0.494 0.845	0.90
4	3,841	3.1	+	0	$75$ $(37.5 \times 2)$	8,750 7,300	+	45 77	0.525 1.050	0.89
	4,120	0.5	-	0	$(37.5 \times 2)$ $(37.5 \times 2)$	3,131 3,010	_	21 34	0.673 1.121	0.87
5	4,210	2.2	+	0	$(37.3 \times 2)$ (50) $(25 \times 2)$	3,010 3,120	+	6	0.199 0.352	0.95
6	2,100	2.5	+	0	50	3,002	+	5	0.167 0.254	0.96
7	5,751	4.1	+	0	$(25 \times 2)$ 50 $(25 \times 2)$	3,502 5,125 5,000	+	10	0.195 0.382	0.90
	4,106	0.5	-	0	50 (25×2)	5,099 3,135 3,861	_	6	0.192 0.398	0.88

Using this model system, we have elucidated the doseresponse relationship for transformation by single acute doses of X rays between 1 and 600 rad<sup>2</sup>. The overall shape of the curve resembled that commonly observed for the production of tumours in experimental animals<sup>7</sup>, namely a rising transformation rate with doses from 1 to 150 rad, followed by a plateau between 150 and 300 rad, after which a further increase of dose resulted in a decrease in the proportion of colonies transformed. This decrease coincided with doses such that the number of

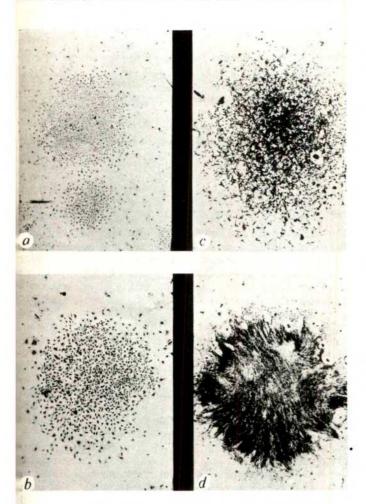


Fig. 1 a and b, 9-d-old clones of normal embryo cells (Giemsa × 10.6); c and d, two representative 9-d-old clones of hamster embryo cells transformed in vitro by a split dose of 75 rad (Giemsa × 10.6).

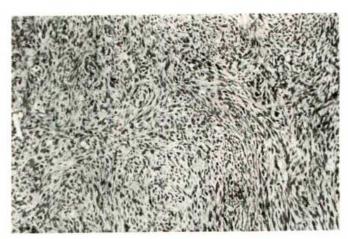


Fig. 2 Histological section of a non-invasive, non-metastasising fibrosarcoma growing in a 3-week-old hamster 10 d after a subcutaneous injection of 10<sup>6</sup> transformed cells obtained as follows. A transformed clone, of the type illustrated in Fig. 1c, produced by X irradiation of single cells, was isolated and grown up through 30 passages before inoculation (haemotoxylin and eosin × 106).

cells killed began to be significant. Our system inherently accounts for cell killing, however, because transformation is scored from among surviving colonies. Consequently, potentially transformable cells seemed more sensitive to the lethal effects of the accumulated damage at the higher doses.

We next pursued the problem of dose protraction since the problems of risk estimates for radiation are related to increments of low doses protracted over long periods, rather than a single acute exposure. For this reason, a single dose of 50 rad was compared with two doses of 25 rad 5 h apart. The doses were chosen to be on the ascending portion of the dose-response curve, while the time interval was a compromise, being long enough to allow for the repair of sublethal radiation damage, but short enough to minimise the probability of cell division between exposures. Plates receiving the single dose were exposed at a time mid-way between the delivery of the first and second doses to the plates. In later experiments the effect of a dose of 75 rad was compared with that of two doses of 37.5 rad 5 h apart.

The results are presented in detail in Table 1, summarised in Table 2 and plotted in Fig. 3. For both 50 and 75 rad, there is a clear increase in the proportion of transformed clones for doses delivered as two fractions compared with the same doses given as single exposures.

	Table 2 Po	oled data	
	Clones counted	No. transformed	% Transformation
Control	38,210		mannaer
75 rad	25,882	160	0.62
$(37.5 \times 2)$	23,762	255	1.08
50 rad	14,272	27	0.19
$(25 \times 2)$ rad	15,582	55	0.35

To check whether the presence of feeder cells was essential for the production of transformed clones, some cells in these experiments were plated on feeder layers and others were not. While the feeder layer (at levels of  $2 \times 10^4$  cells per 60-mm Petri dish) improved plating efficiency, it did not affect the proportion of cells transformed by the radiation.

At these low doses less than 10% of cells were killed, as can be seen from the surviving fractions listed in Table 1. It is also evident from Table 1 that more cells survived split doses than single doses, which shows that sublethal damage was repaired, as in almost every biological system studied.

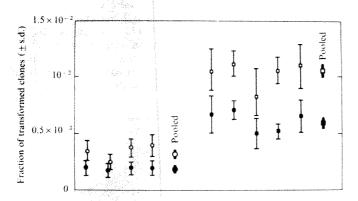


Fig. 3 Incidence of cell transformation in cloned cultures of hamster embryo cells. Four replicate experiments were performed in which the effect of a single dose of 50 rad (●) was compared with that of two doses of 25 rad (○). The results of the individual experiments are shown, together with the pooled data; vertical bars are standard deviations. The consequence of a single dose of 75 rad ( ) compared with two doses of 37.5 rad ( ) are shown.

The results suggest that even at these low doses there is a strong correlation between the proportions of cells killed and transformed. When a dose of radiation is delivered as two fractions fewer cells receive lethal injury, and therefore more cells sustain sublethal damage than when the same dose is delivered in a single exposure. Since X-ray transformation presumably involves sublethal events, it is not surprising that splitting the dose leads to less killing but enhanced transformation.

Comparison of our in vitro data with similar studies of tumour induction in vivo is interesting, although the latter show no clear-cut pattern. Most studies of tumour induction in animals have involved larger doses and longer time intervals than used in our study, and leukaemias and lymphomas rather than solid tumours. Kaplan and Brown<sup>8,9</sup> found that 475 r., given to mice as four exposures at 4 or 8-d intervals, was more tumorgenic than the same dose given as a single exposure or in daily fractions. By contrast, Metalli et al. 10 concluded that split doses of sublethal irradiation with short fractionation intervals are less efficient inducers of tumours and leukaemias than a single dose. Upton et al. found a complex pattern of X-rayinduced myeloid leukaemia in mice11 that was intermediate between these two sets of results.

Our demonstration of an increased incidence of transformation when the dose is divided, albeit with an in vitro system, is germane to the problems of radiation protection. When radiation workers and the general public are exposed to ionising radiation it is usually in the form of multiple small doses rather than a single large dose, as commonly used for experimental studies with small animals. For most end-points observed, including cell killing, fractionation reduces the effect of a given dose of radiation, but if the opposite is true for transformation ours will be an important observation in practical terms.

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### Association of polyanion resistance with tumorigenicity and other properties in BHK/21 cells

QUIESCENT, non-growing cultures of normal fibroblasts may arise as a result of absence of anchorage1.2 or deprivation either of serum macromolecules3-5 or of a variety of nutrients8. This state can also be induced by sulphated polysaccharides", and we have reported the inhibition of growth of normal BHK/21 hamster fibroblasts in monolayer by dextran sulphate and heparin9 and the protection against this inhibition by purines10. Goto et al. have also found that dextran sulphate added to grow ing cultures markedly reduces the saturation density of established lines not transformed by viruses, but not that of primary embryonic fibroblasts<sup>11</sup>. The ability of many cells to grow in agar only after transformation with tumour viruses has been similarly ascribed to the inhibitory effects of sulphated polysaccharides8,12 and a close quantitative relationship has been reported between tumorigenicity and plating efficiency in agar<sup>18</sup>.

. In the light of these findings and the known existence of sulphated polysaccharides and proteoglycans in connective tissue, it is appropriate to seek a role for such polyanions in growth control in such tissues. We have obtained dextran sulphate resistant cultures of normal hamster BHK/21 cells and studied their properties. The results show that there is a close correlation between resistance of clonal sublines to dextran sulphate and a number of other properties: high

Table 1 Clonal variants of BHK/21 cells (see text) compared with parent untransformed and transformed with polyoma virus (PY)

	•	,	• • • • • • • • • • • • • • • • • • • •				
Sept.	Inhibitory cells	$TD_{\mathfrak{s}_0}$	Inhibitional concentration dextran sulphate (µg ml <sup>-1</sup> )	Serum dependence	Attachment	Orientation	
	BHK/21 'S'-type variant 'I'-type variant 'R'-type variant PY BHK/21	$ 3 \times 10^{4} - 10^{5}  3 \times 10^{4} (1)  10^{2} - 10^{3} (12)  10^{2} (9)  10^{2} $	<3 3 (1) 4-32 (8) > 100 (24) > 100	+ + - -‡	+++++++++++++++++++++++++++++++++++++++	+ + + +	

Figures in brackets refer to number of sublines tested. Tumorigenicity was tested by injecting 0.5 ml cell suspension subcutaneously into young adult hamsters (four animals per suspension) containing 10<sup>2</sup>, 10<sup>3</sup>, 10<sup>4</sup>, 10<sup>5</sup> cells; animals were observed for 5 months. The inhibitory concentration of dextran sulphate was that which prevented the doubling of cells in 4 d (Fig. 1). Serum independence is the ability to grow continuously in serum-free HEMP medium (E4, 4 volumes; Hams F12, 1 volume; Pirts, 1 volume); methocel<sup>10</sup> (4,000 c.p.s. Dow Chemical Corp.) to 0.2%; dextran sulphate to 10 µg ml<sup>-1</sup>.

\*Cells pile up in the centre of large colonies, but show orientation at periphery. †Cells attach initially but later grow in suspension. ‡Clarke, G. D., and Ryan, P. R. (unpublished) and Bush<sup>18</sup>.

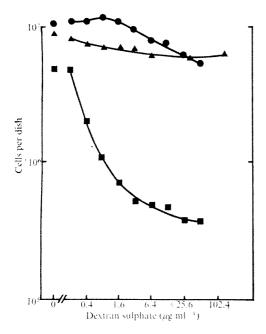


Fig. 1 Cells were grown in duplicate dishes of E4 medium containing 5% serum from an inoculum of  $3\times10^{6}$  per 5 cm dish at 37° C for 4 d. After removal with trypsin versene they were counted with a Coulter Electronic Counter. , Original BHK/21 cells; , BHK/21 cells transformed with polyoma virus and, A, clonal subline 'R-type'. Points are means of duplicates.

tumorigenicity, serum independence and growth in suspension. While the growth of normal BHK/21 cells is completely inhibited by 2 µg ml<sup>-1</sup> dextran sulphate, that of polyoma transformed cells is only marginally affected by 50 µg ml<sup>-1</sup> (Fig. 1).

To select for resistant variants, therefore, normal cells were seeded at 3  $\times$  10<sup>5</sup> per dish in medium containing 5% serum and 0.5 μg ml<sup>-1</sup> dextran sulphate. Dishes were incubated at 37° C until a dense culture was obtained. Subculture was then made at one-tenth dilution into both 0.5 and 1.0 µg ml<sup>-1</sup> dextran sulphate. This regime of subculture at twofold higher concentrations was continued reverting to the same concentration only when inadequate growth was obtained at the higher, until cells were growing vigorously at 16 µg ml<sup>-1</sup> of dextran sulphate. Growth was continued in this medium with progressively decreasing inocula until cells grew reproducibly from 104 per 9 cm dish. This complete selection process was repeated three times and, on two occasions, the cells were then cloned in dextran sulphate-free medium with 10% serum (plating efficiency 25–30%). In the course of each selection it was noted that giant cells appeared, most of which floated or remained loosely attached to the dish; aliquots from the supernatant were always, therefore, incorporated in subcultures. After a few passages in dextran sulphate the cells lost the distinctive oriented appearance of BHK/21 and, in later passages, many remained rounded with apparently healthy cells appearing in suspension. This behaviour was also shown in dextran-free medium. From the resistant cells colonies produced were classified in three groups. Most colonies were composed of spread cells initially and showed central piling up. Sublines from such clones ('I-type') showed variable resistance to dextran sulphate and 102-103 cells injected into hamsters formed tumours in 50% of animals (TD<sub>50</sub>). One colony resembled the original cells, showing oriented growth and full sensitivity to dextran sulphate ('S-type').

Table 2 Interaction of BHK/21 cells and dextran sulphate					
Dextran sulphate in medium A (µg ml <sup>-1</sup> )	Serum in medium B (%)	% Labelled nuclei			
0	0	2			
0	3	53			
20	3	32			
200	3	22			
2,000	3	15			

BHK/21 cells seeded at  $3 \times 10^5$  cells per 5 cm dish in E4 medium containing 0.5% serum and incubated for 3 d. Medium was removed, duplicate dishes treated at room temperature for 1 min with either E4 or E4 + dextran sulphate (Medium A). Dishes were then washed with E4 (two, three and four washes were used and results were not significantly different) and incubated for 20 h with E4 medium or E4 + 3% serum (Medium B) and 3H-thymidine at 1 μCi ml<sup>-1</sup>. Following autoradiography the percentage of labelled nuclei was calculated from 300 in each of four dishes per set.

Nine colonies ('R-type') consisted almost entirely of rounded cells and sublines from these all had a TD<sub>50</sub> as low as that of polyoma transformed BHK/21 cells and were also as resistant to dextran sulphate (Table 1 and Fig. 1). It was further found that all nine sublines were capable of continuous propagation without serum in a complete synthetic medium. A further 15 'Rtype' sublines were established from clones arising in medium containing 16 µg ml<sup>-1</sup> of dextran sulphate (plating efficiency 0.5-2%) all of which grew continuously in serum-free medium. Temin suggested that sulphated polysaccharides might act by combining with serum growth factors and indeed a small precipitate containing protein occurs in dextran sulphate medium and adheres to the dish (R. C. Hallowes, unpublished). Our results, however, indicate that quiescent BHK/21 cells can also react with dextran sulphate in the absence of serum in a manner that, even after thorough washing, inhibits their response to serum (Table 2), indicating that it also binds to the

In view of the tendency of dextran sulphate resistant cells to grow in suspension and the observation of floating cells in dextran sulphate containing medium some hours after seeding, the effect of the polyanion on attachment of cells to dishes was

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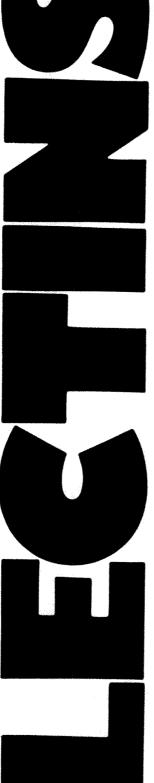
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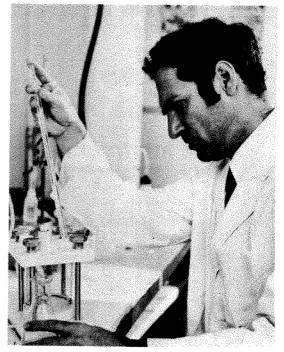
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Table 3 Attachment of BHK/21 and PY cells in dextran sulphate

Serum	Dextran sulphate	Cells att	Cells attached (%)		
%	(µg ml <sup>-1</sup> )	BHK	PY BHK		
0	0	97	98		
0	4	97	97		
0	. 16	98	96		
0	64	97	97		
5	Ó	83	71		
5	4	36	14		
5	16	35	~ <u>`</u>		
5	64	32	ī		
-	• • •		-		

Cells were removed from a culture in E4 medium containing 20% calf serum with 0.05 % trypsin (Difco 1:250) in 0.1 % versene, washed with medium and resuspended in the same medium containing only 5 mM NaHCO<sub>3</sub> and with 30 mM HEPES buffer adjusted to pH 7.0. Aliquots of the same medium (5 ml) were prepared with serum and dextran sulphate as indicated and warmed to 37° C. Cells were seeded into these at  $6\times10^{6}$  each and 2.5 ml of each suspension dispersed into duplicate dishes in a 37° C room. After 20 min the supernatant medium was gently poured off into plastic pots. The dishes were then gently washed with a further 2.5 ml of medium containing 10% serum and the washings added to the pots. Cell numbers were counted in each suspension with a Coulter Electronic Counter and the numbers attached calculated.

examined; Table 3 shows that the attachment of both normal and transformed cells is inhibited in the presence of serum. This behaviour is in line with the observation that mouse L cells in monolayer culture detach from the dish within 10 min on the addition of dextran sulphate<sup>14</sup>.

It is premature to attempt a detailed understanding of the mechanism of inhibition by, and resistance to, dextran sulphate. The simplest hypothesis, that polyanions by competition for cell attachment sites inhibit anchorage which is essential for growth of normal but not transformed cells, is untenable. The inhibition of attachment of BHK/21 cells by dextran sulphate in short term experiments described in Table 3 demonstrates a delay but within a few hours the majority of cells are attached and spread. Moreover, in the absence of polyanions, such cells can grow in suspension.

Our observations with these variants suggest that resistance to dextran sulphate is correlated with a tendency to grow in suspension, independence of serum growth factors and high tumorigenicity. We postulate that dextran sulphate, which is known to react with many proteins<sup>15</sup>, binds to the cell membrane so as to block the interaction with the macromolecular growth factors in serum and to block temporarily the interaction with the solid substrate. The membrane functions involved are probably required for the growth of normal but not that of transformed fibroblasts. The selection process in dextran sulphate under which resistant cells emerge may thus be considered as comparable with that under which malignancy emerges in vivo. It is not yet clear whether the process is analagous, namely whether sulphated polyanions of connective tissue mediate the control of cell proliferation in those tissues. Meanwhile it is important to know whether the phenomena reported here can be observed also in other cell systems.

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### Antibody-dependent cell-mediated damage to schistosomula in vitro

ANTIBODY-DEPENDENT cell-mediated cytotoxic effects have been described in many in vitro models involving single-cell suspensions of maminalian or avian target cells, and could be important in the rejection of tumours and allografts<sup>1-6</sup>. Similar responses could also be involved in protective immunity against metazoan parasites, and evidence for cell-mediated effector mechanisms of various types has emerged from work on Nippostrongylus braziliensis<sup>7</sup> and Litomosoides carinii<sup>8</sup> in rats. Here we present two new aspects of cell-mediated responses to metazoan parasites. First, we have adapted the 51Cr-release technique to assay damage to schistosomula of Schistosoma mansoni in vitro. Second, we provide evidence that normal human peripheral blood leukocytes, in the presence of sera from patients infected with S. mansoni, can damage labelled schistosomula in the absence of added complement.

A naturally-infected field population of Biomphalaria pfeifferi was used to initiate a laboratory colony of S. mansoni, which was subsequently maintained in locally collected snails and Swiss albino outbred mice. Schistosomula were prepared from cercariae by a modified skin penetration technique9, and were stored at 4°C overnight in Hanks' balanced salt solution containing antibiotics and lactalbumin hydrolysate 0.5%, together with 10% heat-inactivated normal baboon serum. This storage procedure was not associated with a detectable loss of schistosomula or decrease in viability, as judged by motility and ability to metabolise fluorescein dibutyrate<sup>10</sup>. After overnight storage, most of the supernatant fluid was removed, and the organisms, after resuspension in the residual supernatant, were labelled with 51Cr-sodium chromate (Radiochemical Centre, Amersham). Incubation of 104 organisms with 0.5 to 1 mCi of <sup>51</sup>Cr in a volume of less than 0.5 ml for 3-4 h at 37° C gave count rates of 50-300 c.p.s. per 100 organisms. Labelled schistosomula were washed five times in HEPES-buffered Eagle's minimal essential medium (MEM) with antibiotics, and were resuspended at 500 or 1,000 organisms per ml in MEM containing 10% heat-inactivated foetal calf serum (MEM/FCS).

Serum samples were obtained from patients with a long history of presumptive exposure to S. mansoni and with eggs detectable in varying numbers in the faeces by the Kato technique11. Control samples were taken from laboratory workers with no known exposure to S. mansoni. All sera were heatinactivated and were diluted in MEM/FCS before testing. Peripheral blood leukocytes were prepared from uninfected human subjects by defibrination and centrifugation over Ficoll-Hypaque<sup>12</sup>. Interface cells were washed twice in MEM and resuspended in MEM/FCS at varying cell concentrations. Such preparations contained both polymorphonuclear and mononuclear leukocytes.

The cytotoxicity test was carried out in a total volume of 0.3 ml in 38×7 mm sterile plastic tubes, incubated in humidified plastic boxes. At the end of the incubation period, 0.15 ml of the

Table 1 Release of <sup>51</sup>Cr from schistosomula after incubation with various sera in the presence of absence of normal human peripheral blood leukocytes

			Isotope re	elease (%)	*		
	Wi	th serum +	cells†	W	ith serum a	lone†	
Serum dilution‡	1:6	1:24	1:96	1:6	1:24	1:96	
Serum							
A40/1 infected	54	48	39	24	22	24	
A39/1 infected	<i>51</i>	49	44	26	26	22	
A39/4 infected	50	51	48	25	23	22	
A38/3 infected	<i>53</i>	49	43	26	23	25	
AEB control	28	29	24	23	23	24	
LTW control	27	27	27	23	23	23	
SN control	26	28	27	22	24	21	
GK control	29	28	25	22	23	23	
Medium§ —		27			$\tilde{24}$	******	

Statistical summary: analysis of variance, 'cells'  $\times$  'serum' interaction, P < 0.001 (F = 43.4: 8 and 162 d.f.); multiple range tests: Values in italics are significantly different from the 'medium with cells' control (P < 0.05).

Final dilution of serum in the cytotoxicity assay mixture.

supernatant was removed, and both cell and supernatant tubes were counted for <sup>51</sup>Cr. Four replicates for each group were set up, and results are expressed as the geometric mean of the original percentage isotope release values. The logarithmically-transformed data were tested by multifactorial analysis of variance followed by Duncan's multiple range tests<sup>12</sup>, and a summary of the statistical analysis is given for each set of data.

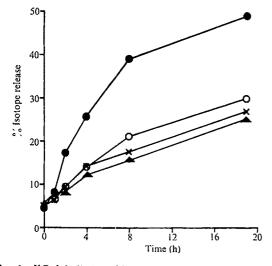


Fig. 1 <sup>31</sup>Cr-labelled schistosomula were incubated for varying periods of time in the presence of: ▲, medium alone; ×, serum alone (A39/1 at 1/30 final concentration); ○, cells alone (multiplicity of 2,000:1); ④, cells+serum. Analysis of variance: 'effector preparation' × 'time' interaction, P<0.001 (F=4.54:15 and 72 degrees of freedom). Multiple range tests: 'Cells+serum' different from other preparations at 2, 4, 8 and 19 h (P<0.05); 'Cells alone' different from 'medium alone' at 8 h (P<0.05).

The time course of isotope release in the presence of various combinations of serum from an infected patient and normal cells is shown in Fig. 1. Release in the presence of medium alone was slow, amounting to only 25% after 19 h. Antibody alone, at a final concentration of 1:30, caused no significant increase in isotope release. Cells alone, at a multiplicity of 2,000:1 to the target organisms, caused a slight increase in isotope release. This multiplicity was chosen arbitrarily, and it should be borne in mind that the target is a large multicellular organism measuring approximately  $400 \times 100~\mu m$ . In later experiments, significant cytotoxicity by cells and antibody was found at multiplicities down to 100:1. Cells in combination with antibody caused a marked increase in isotope release which was

significantly different from the effect of cells alone from 2 h of incubation onwards. By 19 h, isotope release in the presence of cells and antibody was beginning to show a plateau effect at approximately 50%, and in separate experiments it was observed that only 50–60% of the total isotope incorporated could be released by repeated freezing and thawing in hypotonic solution, a procedure which killed 100% of the schistosomula. This presumably reflects the multicellular nature of the organism, which may be resistant to complete disruption. The relationship between isotope release and death of the organism was difficult to determine, but incubation of various preparations of schistosomula with fluorescein dibutyrate<sup>10</sup> and subsequent examination by fluorescence microscopy showed that the percentage of isotope released in a preparation was approximately related to the proportion of organisms that were immobile and that failed to metabolise the fluorescein ester.

The data in Fig. 1 were derived from a single serum sample at a single dilution. Table 1 shows the results of an experiment in which four sera from infected subjects and four sera from control subjects were tested at three dilutions. In separate experiments, a total of eleven test sera and four control sera were examined. None of the sera was toxic for schistosomula in the absence of added leukocytes. All the test sera showed significant effects in the presence of added cells, in seven cases at dilutions down to 1:96. Later examination of three of the positive sera (A40/1, A38/3 and A39/1) showed that significant effects could be obtained at dilutions down to 1:320. None of the control sera showed a significant effect, even at concentrations as high as 1:6.

These results indicate that schistosomula can be damaged in vitro by normal human peripheral blood leukocytes together with sera from infected patients, but not with sera from control subjects. Unlike "lethal antibody" and granulocyte-mediated damage to opsonised organisms<sup>13</sup>, both of which depend on the presence of large amounts of added complement, the effect described here is complement-independent, in that heat-inactivated sera were used throughout. An analysis of the antibody and cell types responsible for eliciting the effect will be published elsewhere (A. E. B., unpublished): preliminary observations indicate that maximum cytotoxic activity is associated with an eosinophil-rich polymorphonuclear leukocyte fraction.

The relevance of the observations to resistance to reinfection cannot at present be established, particularly as it has not yet been demonstrated clearly that human patients do develop an effective immunity to *S. mansoni*. Recent evidence in experimental schistosomiasis in rats, however, has shown that resistance to infection can be transferred to normal animals by injection of immune serum, but that irradiation of immune rats

<sup>\*</sup> Measured after 14 hours.

<sup>†</sup> Schistosomula were cultured either with or without effector cells at a multiplicity of 2,000:1.

<sup>§</sup> Schistosomula cultured with or without effector cells, without added serum.

on the day of cercarial challenge abolishes immunity<sup>14</sup>. These findings suggest that both antibody and a radiosensitive cell may be involved in resistance in rats. Our results indicate that a similar mechanism could be effective in man, and provide a technique for analysing the response in more detail.

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#### C3 activation and T-independent B cell stimulation

Some highly polymerised immunogens with repeated antigenic determinants, such as bacterial polysaccharides or polymerised flagellin and hapten conjugates of these, can stimulate mouse B lymphocytes to make antibody without requiring the cooperative helper activity of T lymphocytes1. The mechanism whereby such T-independent immunogens, after interaction with the surface receptors on specifically responsive B lymphocytes, trigger these into activity is uncertain. Dukor and Hartmann<sup>2</sup> have postulated that binding of activated C3 to complement receptors on B cells acts as a necessary second signal for antibody production. They envisage that T-independent antigens and B-cell mitogens generate this second signal by their ability to activate C3 through the 'bypass' pathway, whereas in the case of T-dependent antigens the signal derives from C3 cleavage by proteases released by activated T cells.

Recent work in our laboratory suggests that neither high epitope density3 nor polyclonal mitogenicity17 are adequate explanations for T cell independent immune induction. Here we extend these observations by examining the correlation between the immunogenic properties of various antigens tested in vivo and their capacity to activate mouse C3 in vitro. On the basis of the above model, all T-independent antigens should be able to activate C3.

Fresh C3H/He mouse serum was used as a source of C3 and the origins of the antigens are listed in Table 1. Sheep anti-mouse C3 serum was made by a modification of the method of Mardiney and Müller-Eberhard<sup>12</sup>. For C3 cleavage, 0.05 ml of the test sample in saline was added to 0.1 ml of fresh mouse serum diluted with an equal volume of Veronal buffered saline12 containing 0.025 M MgCl<sub>2</sub> and 0.025 M ethyleneglycol bis-(aminoethyl)-tetraacetic acid (EGTA) (Koch-Light). The mixture was incubated at 37° C for 45 min. The presence of cleavage products was detected by antigen-antibody crossed electrophoresis<sup>14</sup> in agarose gel containing 0.01 M EDTA and sheep anti-mouse C3 serum.

The presence of EGTA and Mg2+ during incubation provided conditions for activation of complement by the 'bypass' mechanism only<sup>15,16</sup>, and excluded any activation by the Ca<sup>2+</sup>dependent classical pathway because of pre-existing antibody reacting with the antigen. The sensitivity of the method for detecting activation of C3 was tested using various concentrations of Escherichia coli 0.55:B5 lipopolysaccharide (LPS) (Difco) and levan (Fig. 1). LPS was detectably active at concentrations down to 2 µg ml<sup>-1</sup> (although this is not visible in the photograph) and obvious activation was obtained at 4 μg ml<sup>-1</sup>; the preparation of levan was clearly active at 1 µg ml<sup>-1</sup> and some activation was detected at the lowest concentration tested  $(0.5 \mu g ml^{-1}).$ 

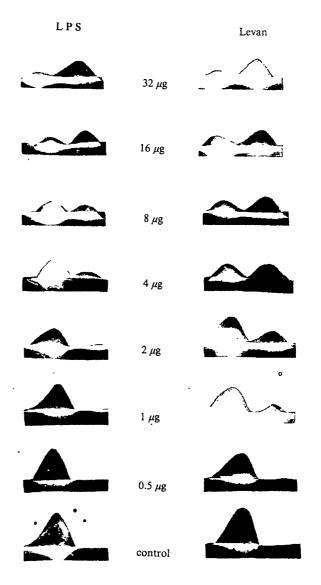


Fig. 1 Comparison by antigen-antibody crossed electrophoresis of C3 activation by various doses of *E. coli* 0.55:B5 LPS and levan. The left hand peak is native C3, the right hand (faster moving) its cleaved product (anode on the right).

Table 1 C3-activating properties of antigens and mitogens

Zubic i Ob don'tding properties of di		~84110
	Concentration	C3
including origin t	tested ( $\mu$ g ml <sup>-1</sup> )	activation
LPS <i>E.coli</i> 0.111:B4 (Difco)	400	+
LPS <i>E.coli</i> 0.55:B5 (Difco)	400	+
• • • • • • • • • • • • • • • • • • • •	200	+
Acid hydrolysed LPS E.coli 0.55:B5 (ref. 4)	400	
Alkali hydrolysed LPS E.coli 0.55:5B (ref. 4)	400	
Hydroxylaminolysed LPS E.coli 0.55: B5 (ref	(.5) 400	
LPS Salmonella mR 345 (ref. 6)	400	
LPS Salmonella TV-119 (ref. 6)	400	+
LPS Serratia marcescens (ref. 7)	400	+
LPS Shigella shigae (ref. 8)	400	+
*CoF	10	+
	5	÷
†CoF	5 units	+++++
•	1 unit	+
‡SIII	400	*****
\$SIII	1,000	
	400	*****
Levan (from Aerobacter levanicum) (ref. 11)	400	+
,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	10	÷
DNP-lys-SIII (ref. 10)	400	,
DNP-lys-levan (ref. 11)	400	+
	10	<u> </u>
¶Hyaluronic acid (Hy)	400	-
¶DNP-lys-Hy	400	
Polyglutamic acid (PGA)	400	-
DNP-lys-PGA	400	
DNP <sub>s</sub> BSA (ref. 3)	400	
DNP <sub>50</sub> BSA (ref. 3)	400	+
	10	<del>-</del> + +

<sup>\*</sup>Cobra venom factor was prepared from Naja naja (ref. 9)

Most of the antigens and mitogens were tested at a relatively high concentration (400 µg ml<sup>-1</sup> of incubated mixture). Some were also tested at lower and higher concentrations as shown in Table 1. Cobra venom factor (CoF), various preparations of LPS (except from Salmonella mR 345), levan and DNP 50 BSA activated C3. Type III pneumococcal polysaccharide (SIII), hyaluronic acid, polyglutamic acid and DNP, BSA were inactive. DNP conjugates of various polymers (which are all immunogenic with respect to the DNP moiety11,17) behaved in the same way as the polymers themselves. Treatment of LPS with mild acid or alkali which abolishes its mitogenicity (G.G.B.K., unpublished) also abolished its C3 activating properties.

Table 2 lists the C3 activation produced by various antigen preparations whose mitogenic activity has been examined in the presence of foetal calf serum<sup>17</sup>. There is no apparent correlation between mitogenic and C3 cleaving activity. For example, levan which activates C3 very efficiently (Fig. 1) is a less potent 'mitogen' than SIII which did not activate C3 (Table 1).

In these experiments the effect of various T-independent anti-

Table 2 Comparison of C3-activating and mitogenic properties of various polymers

C3 activation	Mitogenicity* <sup>3</sup> H-thymidine uptake (c.p.m.×10 <sup>-4</sup> ml <sup>-1</sup> )
+	5.6
+	• 9.3
	1.36
+	0.72
Acres	1.32
	0.52
	0.32
	+

<sup>\*</sup>Cell proliferation in normal mouse spleen cell cultures induced by optimal stimulatory doses, and assayed by uptake of <sup>3</sup>H-thymidine, cells grown in serum-containing medium (data taken from ref. 17).

gens on mouse C3 was examined, as their immunogenic and mitogenic properties have been previously tested in this species. Earlier observations that LPS and some other polysaccharides activate C3 by the 'bypass' mechanism in various species 18-20 are confirmed.

Regarding the significance of C3 activation for T-cell independent B cell triggering however, two points may be made. First, the capacity of an antigen to activate C3 does not necessarily make it T independent. For example, Klaus and Cross<sup>3</sup> have shown that DNP<sub>50</sub>BSA elicits a T-cell dependent IgM response; yet DNP<sub>50</sub>BSA activates C3 effectively (Table 1). Furthermore, the C3 activating CoF itself, which readily elicits antibody in intact mice (and for that reason normally becomes ineffective within 4-5 d) has a prolonged action and fails to elicit detectable antibody in thymus deprived mice (J.P. and J.H.H., unpublished).

Second, the capacity to activate C3 by the 'bypass' mechanism, at least as measured by cleavage of C3 in vitro, does not seem to be an obligatory feature of T-independent immunogenicity (Table 1). Nevertheless our preparation of DNP-lyslevan, which activates C3, is considerably more immunogenic in C3H mice than DNP-lys-SIII, which does not. Similarly, native LPS is a more potent immunogen than LPS treated with acidor alkali (G.G.B.K., unpublished). (In the latter case, however, the comparison is complicated by the fact that native LPS is a potent polyclonal mitogen.)

It seems that neither activation of fluid phase C3 nor 'nonspecific' (mitogenic) activation<sup>17</sup> can be essential prerequisites for specific B cell triggering. The capacity of an antigen to activate fluid phase C3 and/or to cause polyclonal B-cell proliferation, however, may well enhance its intrinsic immunogenicity. Furthermore, it is possible that T-independent antigens which do not activate fluid phase C3 may nevertheless do so, when bound at a cell surface. This possibility is being investigated.

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<sup>†</sup>Commercial anticomplementary factor from cobra venom (Cordis Laboratories).

<sup>‡</sup>Pneumococcal polysaccharide from Type III pneumococci (Wellcome, Lot K 5045).

<sup>§</sup>Pneumoccocal polysaccharide from Type III pneumococci (ref. 10). Preparations shown to behave as T-independent antigens.

## Differential recognition by thymocytes of H-2 and non-H-2 alloantigens

In this report we examine the behaviour of mouse thymocytes in the mixed lymphocyte culture. The thymus is believed to contain large numbers of T cell precursors or immature T cells undergoing differentiation before migrating to the peripheral lymphoid areas as mature, immunocompetent cells. Our results suggest that the thymus contains a class of cell possessing the potential to react against products of H-2 and non-H-2 loci, but that mixed leukocyte culture reactivity against H-2 depends on a factor(s) present in normal mouse serum.

Thymus-dependent (T) lymphocytes have long been suspected of playing a major role in cytotoxic reactions against cell-associated alloantigens<sup>1-5</sup>. T-cell mediated cytotoxicity is usually dissociated into two phases: a recognition phase, resulting in clonal proliferation of antigen-activated cells, and a destructive phase, in which activated cells within the responding population demonstrate cytolytic activity against cells bearing the sensitising antigens<sup>6-9</sup>. These proliferative and effector phases have been studied *in vitro* using the mixed leukocyte culture (MLC) and cell-mediated lympholysis (CML) assays, respectively. For most species MLC activation as well as cell destruction are associated with loci of the major histocompatibility complex (MHC). In the mouse, however, MLC activation can be induced by genetic differences at loci segregating independently of the MHC (H-2)<sup>10,11</sup>.

Lymphocyte activation was examined in the sensitive miniaturised mouse MLC assay<sup>12</sup> using serum-free<sup>13</sup> and mouse

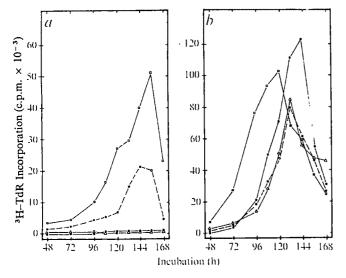


Fig. 1 MLC activation of thymocytes by H-2 and non-H-2 alloantigens in serum-free and mouse serum supplemented media. C57BL/10 thymocytes were mixed with X-irradiated B10.A (△), B10.D2 (●), A/Jax (○) or DBA/2 (□) spleen cells in serum-free (a) or mouse serum supplemented (b) media. 2.0 μCi ³H-TdR were added to each culture 8 h before termination of MLC at the times indicated.

serum supplemented<sup>14</sup> culture media. The genetics of mouse strains used in this study have been described elsewhere<sup>15–18</sup>.

MLC responsiveness of thymocytes from 3-5-week-old mice against H-2 and non-H-2 alloantigens was determined in serumfree and mouse serum supplemented media. Figure 1 shows thymidine (3H-TdR) incorporation for C57BL/10 (H-2b) thymocytes stimulated with X-irradiated spleen cells from (1) B10.A (H-2a) or B10.D2 (H-2d), • two strains possessing genetic differences with C57BL/10 at H-2, and (2) A/Jax (H-2<sup>a</sup>) or DBA/2 (H-2d), two strains differing from C57BL/10 at both H-2 and non-H-2 loci. C57BL/10 thymocytes showed no MLC reactivity against either B10.A or B10.D2 spleen cells in the absence of mouse serum; however, in the presence of serum strong MLC activation occurred. In contrast, C57BL/10 thymocytes responded against A/Jax and DBA/2 spleen cells in both culture media. Similar response patterns have been obtained using numerous strain combinations. Reactions of B10.BR thymocytes mixed with X-irradiated spleen cells from strains with various genetic differences are shown in Table 1. That non-H-2 differences alone are sufficient to induce MLC activation in serum-free medium is demonstrated by the B10.BR-C58 combination.

This differential reactivity of murine leukocytes responding against H-2 and non-H-2 alloantigens in serum-free and mouse serum supplemented media can be demonstrated with thymocytes from 3-5-week-old mice, but is not demonstrable with cells from peripheral blood, lymph node, spleen, or thymus of cortisone-treated mice. These tissues contain few immature cells and are reactive against H-2 alloantigens in either serum-free or serum supplemented medium.

Recent reports 19,20 have shown that various agents—thymus extracts or cyclic AMP—cause differentiation of presumably immature thymocytes into functionally mature T cells. A similar mechanism might also be responsible for the serum requirement in thymocyte responses against H-2 alloantigens. One approach in the examination of this point was to use serum from homozygous nude mice which are athymic and therefore should be deficient in thymus hormone(s). The activity of serum from nude mice (possessing the homozygous nu locus on a BALB/c background) was compared with serum from normal BALB/c mice (Fig. 2). B10.D2 spleen cells or thymocytes were mixed with X-irradiated spleen cells from DBA/2, a strain possessing only non-H-2 differences, or C57BL/10, a strain possessing an H-2 difference, in media supplemented with serum from either normal or homozygous nude mice. The results indicate that B10.D2 thymocytes (Fig. 2a) respond much more strongly against C57BL/10 spleen cells (an H-2 difference) in medium supplemented with serum from normal BALB/c mice than in medium supplemented with serum from nude mice. In contrast, thymocyte responses against the non-H-2 antigens (DBA/2) showed no marked difference in the ability of the two sera to support the mixed reactions. There was little or no difference between the 3H-TdR incorporation by spleen cells responding against H-2 or non-H-2 alloantigens in the two sera (Fig. 2b). These results parallel the serum requirement for MLC activation of thymocytes with H-2 but not non-H-2 alloantigens shown in Figure 1 and Table 1. Note, however,

Table 1	MLC responses of normal thymocytes against H-2 and non-H-2 alloantigens			
Responding strain	Stimulating strain	Genetic difference in MLC combination	Minus serum Mean c.p.m. ± s.d.	Plus serum Mean c.p.m. ± s.d.
	B10.BR C58 B10.A A/Thy-1 B10.D2 DBA/2	None non-H-2 H-2 H-2, non-H-2 H-2 H-2, non-H-2	$\begin{array}{ccc} 547 \pm & 68 \\ 4,318 \pm & 65 \\ 782 \pm & 600 \\ 2,538 \pm & 51 \\ 528 \pm & 23 \\ 12,109 \pm 2,295 \end{array}$	$\begin{array}{c} 315 \pm & 23 \\ 61,748 \pm & 8,134 \\ 711 \pm & 340 \\ 591 \pm & 103 \\ 105,692 \pm & 2,819 \\ 220,766 \pm & 19,470 \end{array}$

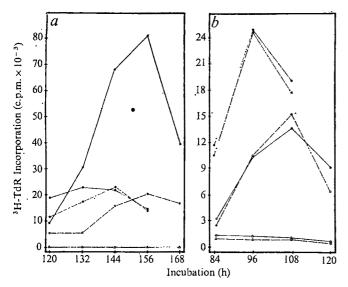


Fig. 2 MLC activation of thymocytes and spleen cells by H-2 and non-H-2 alloantigens in medium supplemented with serum from normal or nude mice. B10.D2 thymocytes (a) or spleen cells (b) were stimulated with X-irradiated C57BL/10 (c) or DBA/2 ( ) spleen cells in medium supplemented with serum from normal (—) or nude (- - -) mice. 2.0 µCi ³H-TdR were added to each culture 8 h before termination of MLC at the times indicated.

that some reactivity occurs using the serum from nude mice in the reaction of B10.D2 thymocytes against C57BL/10 cells.

Results of this study suggest the presence in mouse serum of a factor (or factors) which confers a state of immunocompetence on a class of cell which resides in the thymus of young mice and which is highly reactive against the products of H-2. The serum component permitting mouse thymocytes to respond against H-2 alloantigens is the subject of much speculation, as is the mode of action. Results of experiments showing the effects of serum from normal and from homozygous nude mice on the thymocyte responses to H-2 and non-H-2 antigens strongly supports the concept that this is a 'thymus factor'.

At present, it seems most probable that mouse serum acts on the T-cell precursors or immature T cells of the thymus and induces the state of immunocompetence (for example, development of specific receptor sites on the surface of the T cell). This possibility is supported by studies<sup>20</sup> in which a serum factor secreted by humans and mouse thymus confers on immature cell populations rosette forming capacity or a state of responsiveness to concanavalin A (con A).

In addition to H-2 reactive cells, the thymus also contains a class of cell reactive against products of the non-H-2 loci. On the basis of their MLC responsiveness in the serum-free medium these are probably a different class of cell. It is unlikely that responses against non-H-2 alloantigens in serum-free medium are due to their being more immunogenic than the H-2 alloantigens, because in the serum supplemented medium responses to H-2 are usually stronger than to non-H-2 antigens (Fig. 1).

H-2 alloantigens represent a special class of antigens to the immune system—they serve as highly effective target antigens for T-cell mediated cytotoxicity whereas non-H-2 antigens do not<sup>8</sup>. Other studies (A. B. Peck, unpublished data) show that thymocytes develop cytotoxic potential after their strong MLC activation by H-2 alloantigens only in the presence of mouse serum; no CML is observed in serum-free medium. Thymocytes also fail to develop cytotoxic potential against non-H-2 alloantigens despite strong MLC activation in strain combinations possessing genetic differences at only non-H-2 loci in either medium.

This culture system may define two classes of T cells by their in vitro response patterns to H-2 and non-H-2 antigens, and provides an easy method for identifying T cell functions of the two classes. Studies are in progress to separate and isolate the two cell types.

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#### **Erratum**

In the article "Science and works of art" by J. P. Brommelle and N. S. Brommelle (Nature, 250, 767; 1974) the address given for N. S. Brommelle was incorrect. N. S. Brommelle is at the Victoria and Albert Museum.

#### Corrigendum

In the article "Radiological mapping of the ribosomal RNA transcription unit in E. coli" by P. B. Hackett and W. Sauerbier (Nature, 251, 639; 1974) the authors erroneously referred to the work of Bleyman et al. (J. Bact., 99, 535; 1969) (ref. 20) as being conducted in E. coli. These studies of Bleyman et al. utilised B. subtilis in which the transcriptional order of the ribosomal RNA genes seems to be the same as in E. coli (Pace, N. R., Bact. Rev., 37, 562; 1973) (ref. 1).

# matters arising

### Immunopathy of parasitic infection

THE News and Views article 'Immuno-pathology of Parasitic Infection" by our correspondent F. E. G. C. (246, 187; 1973) has elicited several letters. He wrote "most parasites are capable of evoking immune responses in their hosts but these are seldom effective in eliminating the infection" and in conclusion that "as more information about immunity to parasitic diseases accumulates the possibilities of developing useful methods of immunisation fade further and further into the distance".

• Professor G. M. Urquhart of the University of Glasgow writes: "The first statement is inaccurate and the second is surely a personal opinion largely based on an erroneous extrapolation of the excellent work of Warren¹ who has shown that schistosome egg granulomata are the product of a delayed-type hypersensitivity reaction.

The development of a highly effective degree of acquired immunity both in the sense of the elimination of infection and in resistance to reinfection has been shown to occur in many experimental helminth infections and in domestic animals its natural acquisition plays an essential role in the survival and health of adult cattle, sheep and horses constantly exposed to heavy infection. Over the past 16 yr millions of calves in Europe have been successfully immunised with a vaccine prepared from X-irradiated larvae against Dictyocaulus viviparus, the lungworm of cattle.

The same situation applies to protozoal infections. Antigenic variation does present a problem in artificial immunisation but under natural conditions man and animals survive in large numbers in malarial and trypanosome endemic areas respectively, apparently through the development of an effective immunity. Leishmaniasis in experimental animals and man and coccidiosis in domestic animals also offer excellent examples in which acquired immunity is highly important in protection.

For many years immunological research on many of these important diseases was neglected and we should not be too dismayed if the present in-

terest in parasitic immunology elicits some facts which, at least at first sight, appear disheartening from the viewpoint of the rapid development of a comprehensive range of vaccines."

• Dr D. G. Colley of Vanderbilt University, Tennessee writes: "The final paragraph draws a conclusion regarding immunisation potentials in parasitic infections which, although possibly correct, is based on what I consider to be erroneous reasoning. It reflects a misinterpretation of the basic tenets of either parasitology or immunology, or both. I do not argue with the possibility that the induction of a protective immune response in schistosomiasis may be difficult, or even impossible to achieve. As has recently been pointed out such immune resistance mechanisms have certainly not been convincingly demonstrated and may not even exist.

The article seems, however, to base this assumption upon the demonstrated immunopathogenic effects of an antiegg response. This ignores both the uniqueness of the various intramammalian stages of the schistosomes (schistosomules, adult worms, eggs) and the fundamental concept of immunological specificity. Certainly to induce or increase anti-egg cell-mediated responses could be potentially disastrous (although the induction of enhancing antibodies might be beneficial). Responses against egg antigens have, however, long been seen as of little protective consequence. What the discipline needs, and many investigators are pursuing, is more attention to immune responses against specific antigens."

◆ Dr T. A. Miller, of the Jensen-Salsbery Laboratories, Kansas City, Missouri, writes: "The article should more appropriately have been entitled "Immunopathology of Schistosomiasis". The emphasis on this one host-parasite relationship excludes, for all practical purposes, consideration of the other 95% of parasitic infection. Moreovar, the final statement represents only one aspect of the current international opinion, viz-à-viz schistosomiasis.

As a general statement applicable to the other 95% of parasitic infections, this is patently uninformed and misleading in view of the proven efficacy, safety and widespread use on two continents of irradiated nematode vaccines for lungworm disease of cattle and sheep and hookworm disease of the dog."

<sup>1</sup> Warren, K. S., Trans. R. Soc. trop. Med. Hyg., 66, 417 (1972).

### Population estimates from recapture studies

Bell suggested that P = ((N-a)!(N-n)!)/(N-n)!(N! (N-a-n)!) is the probability that a population is not larger than N in size, where a animals have been captured, marked, and released, and on a subsequent occasion n animals have been captured, all of which have been found to be unmarked. Though P is indeed the probability of the latter event given that the size of the population is N, Bell advances no reason why the probability that the population is not larger than Nshould be equated to it. Indeed, Fisher<sup>2</sup> has stressed it should not: 'The direct step from the test of significance to a probability distribution cannot be sustained, and this circumstance has been responsible for some misunderstanding, and confusion of the terminology.'

Were Bell's treatment valid we could find the probability that the population is of size N exactly by subtraction, yielding

 $p = an \times ((N-1-a)!(N-1-n)!)/(N! (N-a-n)!)$ 

Suppose one marked animal were released (a=1) and subsequently one animal captured and found to be unmarked (n=1). Then p=1/N(N-1), indicating, for example, that N=10 is 110 times more probable than N=100. But most people, I suggest, would feel that they had learnt very little about N (except that it was at least 2), and they would be almost indifferent between N=10 and N=100.

This is reflected in the solution given by likelihood theory<sup>3</sup>, in the context of which the problem is entirely standard. Since P is the probability of what was observed, given N, it is the likelihood of the hypothesis N given what was observed. It attaches a likelihood to each possible value of N, increasing from n!a!/(n+a)! for N = a+n, to unity for N very large. For the case a = n = 1, P = (N-1)/N, indicating that 100 is only 1.1 times more likely than 10.

A minimum estimate of population size is given by the lower 2-unit support limit<sup>3</sup> found by solving  $P=1/e^2=0.1353$  for N. By the very nature of the problem there can be no upper limit, and likelihood theory prescribes none.

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DR BELL REPLIES—Edwards makes two major points: first, that my estimate¹ of population size is strictly invalid; and secondly, that it is of no practical value in situations where its use might be considered.

Although no statistician, I have satisfied myself that Edwards' first point is correct, and that the closing sentences of my original communication must be revised. If no recaptures are made, then clearly P = 1 when the population is assumed to be infinitely large. This does not mean that it is absolutely certain that the population is infinitely large; on the contrary, we may be quite sure that it is not. It means that if the population were infinitely large, we could be quite certain that no recaptures would be made. At some finite value of N, say  $N^*$ , the value of P will be 0.5; that is, if we performed many recapture surveys, obtaining the same sample size on each occasion, we would fail to make any recaptures on about half the total number of occasions. If, therefore, we perform a recapture survey and obtain no recaptures, we still have no evidence that the population is really larger than N\*; and it is in this sense that  $N^*$  is a minimum estimate of  $\dot{N}$ . The value of P chosen depends on how certain we wish to be in setting an upper limit to N\*.

Edwards' second point seems less fully justifiable. The example that he gives involves the very smallest sample that can yield any information\_whatsoever concerning the size of the population, and it is not surprising that only the most tentative conclusions can be formed. With larger samples, useful information can be obtained which is not utilised by standard recapture techniques: in a study of the distribution of population size in newts (G.B., unpublished), the results obtained from the nil-recapture method were found to be entirely consistent with those obtained from two other techniques; stochastic and deterministic recapture surveys, and trap-ratio estimates (Fig. 1). Statistical analysis is primarily a means of interpreting observations of nature, rather than an independent academic discipline. and it is to be hoped that the nil-recapture

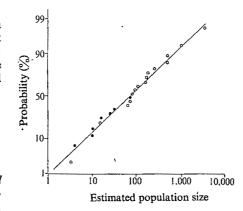


Fig. 1 The distribution of population size in the smooth newt (Triturus vulgaris (Linn)) near Oxford (G.B. unpublished). Each point represents the estimate of the size of a single population. The data are plotted according to the method of Harding<sup>2</sup> for small samples; population size can be seen to be lognormally distributed.  $\bullet$ , Estimates due to the nil-recapture method, using P=0.5;  $\bigcirc$ , estimates from standard recapture techniques, or from a known relationship between the rate of trap-captures and the total population size.

method will be useful, in some circumstances, to biologists working in the field. It may be that its rationale is less rigorous than that of the higher reaches of likelihood theory, but it is at least comprehensible to field biologists without specialised mathematical training. To accept mathematical conclusions whose truth one is unable to establish personally, is to accept the argument from authority—against which all scientific enquiry is a revolt.

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# Protein phosphorylation during oocyte maturation

Morrill and Murphy<sup>1</sup> suggested that "the release of prophase block at ovulation is associated with intense protein phosphorylation" coinciding with 'the activation of a protein kinase (possibly via cyclic AMP)". They further found that "the principal protein species phosphorylated between meiotic prophase and the second metaphase appears to be the phosphoprotein phosvitin". But proper controls for their experiments seem to have been missing. It was not determined whether oocytes that remained in prophase block but were exposed to the same amount of  ${}^{32}P_{1}$  for the same time as those undergoing maturation would also have phosphorylated endogenous protein, as our experience indicates2.3. Nor is it clear that their phosvitin was authentic.

We have repeated their experiments, using an in vitro system. Approximately

120 fully grown oocytes from Xenopus laevis (injected with 1,000 U of human chorionic gonadotrophin 3 weeks preously to flush out over-ripe oocytes) vere dissected manually from their follicles and placed for 2 h in 10 ml of solution O-R2 (ref. 4) containing 1 mCi <sup>32</sup>P<sub>1</sub> (carrier-free, Schwarz/Mann). After 2 h they were washed, and half were placed in 15 ml of solution O-R2 containing 10 µg progesterone ml<sup>-1</sup> to induce maturation<sup>5</sup>. Remaining oocytes were placed in progesterone-free medium (zero time). After 30 min both groups were transferred to medium lacking progesterone. At 6 h, approximately 60% of progesterone-treated oocytes had a diffuse white area centred at the animal pole, indicative of germinal vesicle breakdown5. By 9 h, virtually all treated oocytes had the white area, while untreated oocytes were negative. Dissection of boiled oocytes indicated that the appearance of a white area corresponded with the vesicle breakdown in every case examined (N = 24). Sterile technique was used, 10 µl of antibiotic-antimycotic solution (Grand Island Biological Co.) was added per ml of incubation medium, and the temperature was 20° C.

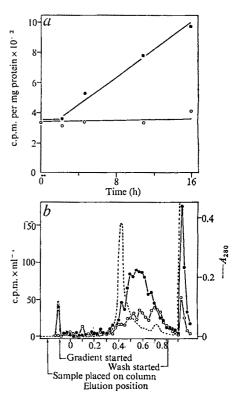


Fig. 1 a, Specific activity of <sup>32</sup>P-protein in progesterone-treated oocytes (●) undergoing maturation and control oocytes (○). Oocytes were preincubated for 2 h before zero time in <sup>32</sup>Pi. Each point represents the average of three determinations. The arrow indicates the time of progesterone treatment. b, DEAE-cellulose chromatography of extracts from oocytes taken at the end of the 16-h period indicated in (a). The dashed line is the absorbance trace; other symbols are as above.

At various times oocytes were placed in water at 100° C. After several minutes, each was transferred to 0.5 ml of 1% sodium dodecyl sulphate plus 5 mM dithiothreitol (pH 8.0) and incubated at 50° C until dissolved. Samples of dissolved oocytes were placed on 23-mm diameter disks of Whatman No. 42 paper, air-dried and treated with cold and hot trichloroacetic acid (containing 1 mM NaH<sub>2</sub>PO<sub>2</sub>) and alcohol and ether to remove low-molecular-weight material, nucleic acid and lipid2. The disks were counted for 32P-protein in a Beckman Lobeta II planchet counter and then used to determine total protein<sup>6</sup>. Figure 1a shows that, after a lag of several hours, the specific activity of the progesteronetreated oocytes increased with time, whereas that of the untreated oocytes did not.

After 16 h, 45 oocytes from each group were homogenised in strong salt solution2, dialysed and chromatographed on a 23/0.9 cm TEAE-cellulose column7. Under these conditions lipovitellin and phosvitin eluted at positions 0.42 and 0.72, respectively. Samples from each fraction were assayed for 32P-protein3 (Fig. 1b). Neither lipovitellin nor phosvitin appeared to be specifically labelled in either case, and the increased amount of labelled material in progesteronetreated oocytes was found (a) initially percolating through the column, (b) between the lipovitellin and phosvitin peaks, and (c) eluting with the wash solution at the end of the run.

We conclude that there is an increase (although not intense) in protein phosphorylation during oocyte maturation, but that phosvitin is not involved. Phosvitin could serve as an energy source during embryogenesis, as suggested1,8. The dephosphorylation observed by Morrill and Murphy during early embryogenesis, however, probably involves some other protein(s). We do not yet know whether the increased phosphorylation during oocyte maturation results from an "activation of a protein kinase (possibly via cyclic AMP)." We have previously described a protein kinase from amphibian oocytes and eggs that is stimulated by cyclic AMP when histone is used as the substrate but not when phosvitin is the substrate<sup>8</sup>.

This research was sponsored by the US Atomic Energy Commission under contract with the Union Carbide Corporation.

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Dr Morrill replies—Dr Wallace appears to confirm our observation1 that release of the prophase block in amphibian eggs is associated with increased protein phosphorylation. The in vitro studies of Wallace and our in vivo studies are not, however, directly comparable. We injected <sup>32</sup>Pi directly into gravid females together with inducing levels of pituitary extract. It is our experience that isolated body cavity (ovulated) eggs take up and incorporate <sup>32</sup>Pi and would probably do so in both the body cavity and oviduct. The in vivo maturing eggs were thus exposed to 32Pi for at least 24 h until released from the oviduct. In contrast, Wallace preincubated isolated oocytes with 32Pi for 2 h and then transferred the oocytes to medium containing progesterone and unlabelled Pi. We have found that intracellular 32Pi of excised oocytes exchanges rapidly with extracellular Pi. Thus, during the progesterone stimulus 32P incorporation into protein would be limited to diminishing endogeneous supplies of phosphorylated nucleotide. This might contribute to the lower overall protein phosphorylation reported in the in vitro system, and possibly to differences in the protein species phosphorylated.

The main difference between our results and those of Wallace is the identification of the phosphorylated protein species. A comparison of a number of phosvitin extraction methods indicated that the major 32P-labelled protein(s) of the in vivo ovulated frog egg could be isolated by the method of Mano and Lipmann<sup>2</sup>. This ovulated egg 'phosvitin' co-electrophoresed with commercial hen's (ovulated, unfertilised) egg phosvitin on SDS polyacryalamide gels. This 'phosvitin' differs from that characterised by Wallace3 in that: (1) it has a lower phosphate content; (2) it is extractable with lower salt concentrations; and (3) it is associated with the cortical pigment granule fraction and not with the yolk platelets. This suggests that amphibian egg phosvitin exists more than one metabolically active form, one principal difference being the level of phosphorylation. In this regard, Mano and Lipmann<sup>2</sup> have shown that fish egg phosvitin can be separated into discrete peaks containing 3.2, 5.7, 7.2, and 9.5 % P. The existence of discrete phosphorylation levels of the same protein backbone structure is particularly interesting.

Neither our maturing oocytes nor Wallace's were compared with the ideal control, that is oocytes completely free of follicle cells. In contrast to the ovulated eggs used in our experiments, oocytes dissected manually as described by Wallace retain a surface layer of intact follicle cells and these cells may also actively phosphorylate protein. When we injected 32Pi into gravid females with no hormone stimulation we recovered 32Plabelled protein in excised ovaries and manually dissected oocytes after 24-48 h in vivo. But, there was no significant 32P incorporation into the 'phosvitin' fraction isolated as described above. This latter information was accidentally omitted from our final reduced manuscript, through the oversight of the authors.

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#### Tin mineralisation

SILLITOE's recent letter1 on tin mineralisation and mantle hotspots is grossly misinformed about the age of the tin mineralisation in South West Africa. The tin deposits do occur in pegmatites but they are unrelated to the possible plume generated Jurassic-Cretaceous alkaline complexes in Damaraland. Instead the pegmatites are related to the Late Precambrian Damara orogen. Radiometric age determinations for a number of pegmatites are in the range 450 to 650 Myr (ref. 2.) I know of no tin mineralisation associated with the Mesozoic alkaline granites in South West Africa and they cannot, therefore, be used to support the contention of a relationship between tinbearing silicic rocks and mantle hot spots.

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### Primary structure of E. coli dihydrofolate reductase

In a recent paper, the primary structure of Escherichia coli dihydrofolate reductase was reported from this laboratory. The paper also suggested a

similarity between a portion of its primary structure and a portion of four vertebrate dehydrogenases.

In a private communication, Drs M. Rossmann and C. Brändén have informed us that the portions of three of the four dehydrogenases chosen for comparison are not from comparable areas of their tertiary structure2-6, and that the statistical treatment given in my paper is inappropriate.

The misalignment of the dehydrogenases does not, of course, affect the primary structure of dihydrofolate reductase shown in Fig. 1 of ref. 1.

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#### All female butterfly broods

Owen, Owen and Chanter<sup>1</sup> have invoked Y-linked meiotic drive to explain the high frequency occurrence of allfemale broods in some populations of the African butterfly Acraea encedon. This mechanism does not account for the following observations<sup>2</sup> of these populations: first, that they only occur in 'disturbed' areas; second, that females aggregate and lay egg batches in close proximity to each other; and third, that females 'court' other females.

The following explanations are advanced for these observations. First, an environmental disturbance (with its consequent effect upon larval food plants) will alter the spatial relationship between egg batches. In mixed sex progeny this may well increase the probability of the first matings being between brother and sister. All-female

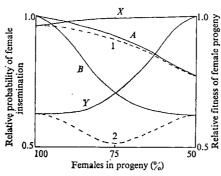


Fig. 1 Net female fitness values (curves 1 and 2) of broods varying in their sex ratio. Curves A and B show a reduction in fitness of females from mixed broods relative to that of all-female broods (as a result of in-breeding). Curves X and Y show the consequent reduction of female insemination probability in all-female broods relative to mixed broods. Net female fitness values calculated by multiplying relative probability of insemination of female progeny by relative fitness of female progeny (curve  $1 = A \times X$ , curve  $2 = B \times Y$ ).

broods do not sustain this disadvantage. Such inbreeding will increase the homozygosity of the genetic environment surrounding the normal Y chromosome and viability loss is a well documented aspect of homozygosity3-5. Therefore the mutant chromosome will sustain an advantage and spread through the population. As it does so, the probability of all-female broods being laid near mixed broods increases and with it the inbreeding probability of these latter broods.

As male frequency drops, however, mixed brood females have a greater probability of insemination<sup>1</sup>. The figure shows that with increased inbreeding the net fitness of all-female broods increases (curve 1) and then reaches equality (curve 2) with respect to mixed broods as male frequency drops.

Second, a mutant female laying its eggs in close proximity to a mixed sex batch will increase the insemination probability of its offspring. The aggregation and egg laying strategies may therefore be accounted for.

Third, any mutant female able to predict oviposition in another female and act as in the second case above will sustain an advantage. This, I suggest, may account for intra female 'courting'.

In non-disturbed (and possibly some disturbed) areas the Y mutant must sustain a net selective disadvantage as evidenced by its non-appearance. The •nature of this selective pressure is not yet known.

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DR CHANTER REPLIES—Over the years, we have received many suggestions as to how the curious population biology of Acraea encedon might be explained, and we welcome another. There are, however, some difficulties with Manning's explanation of the tendency for the occurrence of all-female broods to be associated with 'disturbed' areas. Here I will confine myself to two of these difficulties, both concerned with the figure.

First, the lines on the graph are quite arbitrary, and the different slopes of curves 1 and 2 result from the fact that while curves Y and B have been drawn as reflections of one another (about a vertical line through the 75% point), X and A have been drawn asymmetrically. It would be easy to alter the asymmetry of curves X and A and reach a different conclusion.

Second, to obtain the net fitness of all-female broods relative to that of females from mixed broods (curves 1 and 2), it is necessary to multiply curves X and Y by the reciprocals of curves A and B rather than by curves A and B as they stand, since the relativity in curves A and B is opposite to that in X and Y.

Manning's explanation of the aggregating behaviour is interesting, but would be more convincing if the eggs laid by participating females were fertile-all the eggs we have collected from aggregations have been infertile1.

Finally, I must emphasise that we have not established conclusively that the sex ratio trait is caused by a Ylinked gene, and that we also have some idea as to why the trait is not established in some populations, as explained in our paper2 in which the peculiarities of an isolated population at Gegbwema in Sierra Leone are described. More recently we have discovered a population at Dar es Salaam in which the trait may be just beginning to appear and we hope that this discovery may yield additional information.

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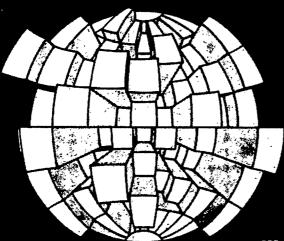
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John Gribbin & Stephen Plagemann



Earthquakes are the most devastating natural phenomenon known to man and have taken an enormous toll of life and property throughout human history. Until now, we have had no accurate means of predicting their occurrence although much effort has been put into research to discover the possible mechanisms which trigger off these cataclysmic events. John Gribbin and Stephen Plagemann present in this book a new model which aims at predicting earthquakes through the study of the hitherto unrecognized and apparently unrelated phenomena of planetary alignments, sun spot activity and the solar wind, and their vital role in determining the course of natural events on earth. These causal links are shown to affect earth movements in active seismic areas and thus unleash the powerful forces which cause earthquakes. Earthquakes are the most devastating natural phenomenon

But their research goes beyond the general model. A particularly unusual planetary alignment will occur within the next ten years and the authors argue a convincing scientific case for major earthquake activity during that period and stipulate the year in question. In particular they show that an earthquake of devastating proportions may well occur along the highly active seismic zone of the San Andreas Fault in California which runs through the cities of Los Angeles and San Francisco. The physical, social and economic implications of a major earthquake in this populous area are far reaching. They make this a book which should be read by all scientists and non-scientists concerned about the effects of natural events beyond the immediate control of man.

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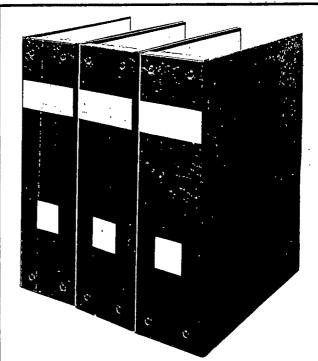
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# reviews

THE publication of this book comes at an opportune time in the development of potentially important applications of superconductivity; a time when important decisions on the extension of existing programmes are under consideration.

Unlike the semiconductors, the future of superconductors seems to be in their application to large scale engineering devices such as high field magnets in particle accelerators, power cables, large machines and generators, and magnetic levitation and linear motor propulsion systems. The basic thinking behind nearly all of these schemes is that they apparently offer the best solutions to many of the economical and social problems facing the advanced countries of the west in such fields as the energy crisis, dwindling natural resources, and pollution and the environment.

Based on discussions that took place in a select NATO gathering of some of the world's leading experts in the fields of superconductivity and low temperature engineering, this book has been biased towards the large scale engineering projects rather than the purely scientific interests. The original conception and subsequent planning of the NATO Institute, which culminated in this publication, resulted almost entirely from the efforts of Simon Foner and Brian Sohwartz of the Francis Bitter National Magnet Laboratory at MIT, who must be warmly congratulated.

Although the phenomenon of superconductivity was discovered by Kamerlingh Onnes as long ago as 1911, until recently it has been extremely slow in making its mark in the real world of practical technology. Only since our understanding of superconducting behaviour and the metallurgy of the type II materials has been established in the last decade or so has the successful manufacture of superconductors been possible. But although operation at liquid helium temperatures has become an engineering routine the real problem still remains, namely, that of introducing the conventional engineer to a new low-temperature environment and convincing him of the reliability and safety of cryogenic devices. This situation has been further aggravated by the huge development costs which inevitably are involved in all such large scale applications. The timely appearance of this book presents the situation

# Towards a new technology

R. G. Rhodes

Superconducting Machines and Devices: Large Systems Applications. Edited by Simon Foner, and B. B. Schwartz. Pp. xx+692. (NATO Advanced Study Institutes Series.) (Plenum: New York and London; NATO Scientific Affairs Division, 1974.) \$47.00.

in exacting detail as it existed in seven different countries up to the end of 1973. It is divided into 17 chapters extending to some 690 pages, contributed by nearly two dozen authors.

The first seven chapters constitute by far the major contribution. Each treats in considerable detail a specific area of technology in which superconductivity is the essential part. The more significant fields of investigation are introduced in the first chapter by J. Powell of the Brookkaven National Laboratory. In particular, he makes a special case for the magnetic levitation of high speed ground transportation systems using the so-called null flux system, an area of applied superconductivity in which he has a special interest. The theory and fabrication of practical superconducting materials are discussed in detail in chapter 2. On the basis of our present metallurgical understanding and experimental research a Tc of 25 K could be realised by 1985. That would truly be a significant breakthrough for cryogenics.

The one important application in which superconductivity has found a permanent niche is that of high field magnets and, more particularly, in the field of high energy physics. The design, construction and behaviour of the more significant magnet systems in the various major laboratories throughout the world are discussed in chapter 3. The following two chapters are devoted to rotating machines. The design details of a superconducting homopolar motor, and its successful application at the Fawley power station, is described. From the results of further experimental projects on d.c. machines it is predicted that the heavy industrial power industry is poised on the threshold of a new era.

Considerable progress has also been made on parallel programmes investi-

gating the application of superconducting windings to a.c. machines, which are discussed at length in chapter 5. The preliminary design calculations indicate that the current density available with high field superconductors may well provide an increase in the economic power density by a factor of 5 to 10 over conventional machines, and may also have a significant volume advantage.

The case made in the first chapter for a mass ground transportation system using superconducting magnets is taken up again in chapter 6, with arguments for an alternative repulsion system and linear synchronous motor propulsion. From exhaustive theoretical studies confirmed by laboratory tests there seem to be no fundamental technical problems which remain to be solved. There are, however, many technical innovations needed before such a high speed transportation system can be put into practical use.

One of the first applications to be seriously examined was the superconducting power cable. With the constantly increasing demand for electrical power, generating stations of ever increasing size are required and, therefore, so are transmission systems of higher capacity and efficiency. The construction and configuration of both a.c. and d.c. cables are fully described in chapter 7. The 12 major superconducting cable projects being undertaken at 10 different laboratories throughout the world are considered to be fully justified by the fact that they represent the most economical solution for power ratings of 2,000 MW or more, which are expected to be the norm in the not too distant future.

Chapters 8, 9 and 10 are supplementary chapters in which three promising areas in the new technology are discussed. The use of hydrogen as an energy carrier is described in chapter 8, followed in chapter 9 by an account of the Josephson junction as a computer device. In chapter 10 a project investigating the use of superconducting magnets for high grade magnetic separation is outlined.

Finally, in seven relatively short chapters from 11 to 17, the essential details of the national programmes in applied superconductivity being undertaken in France, Germany, Italy, Japan, Switzerland, the UK and the US are outlined and details of the institutions, the personnel, and funding

have been included. That innovation in a book of this nature is a welcome addition and, as pointed out in the preface, should lead to better national planning as well as to international cooperation.

This is a book of international status in every sense and there seems little doubt that it should go a long way in promoting a better and wider understanding of this important area of low temperature technology. It should prove to be an invaluable aid to the decision makers in the assessment of future large scale technologies.

# Starting with an open mind

Development in Infancy. (A series of Books in Psychology.) By T. G. R. Bower. Pp. viii+258. (W. H. Freeman: San Francisco and Reading, July 1974.) £5.20 cloth; £2.90 paper.

It was the style of 18th Century philosophers of mind to premise their arguments upon speculative assumptions concerning the original nature and subsequent development of human perception. Bower's splendid little book is in that tradition—but with one hugely crucial difference. In place of speculative assumptions about the original nature of perception the pages of this volume provide a stunning array of experimental research, much of it by the author and his collaborators, designed to explicate how in fact the infant during the opening years of life comes to perceive space, objects, locus and trajectory, and how he develops appropriate concepts and behaviours to cope with what he comes to know. Bower has given us not only a compendium of empirical findings, but a speculatively (sometimes too speculatively) argued treatise on the starting nature of human knowledge and the course of its early transformation in response to encounters with the environment.

It may well be the most important work on early human development since the launching of Piaget's magisterial research a quarter of a century ago, and although it brings that work into question both broadly and in detail, it builds upon it as a starting point. For it is not the work of the Geneva group that is brought most deeply into doubt by this book, but rather the empiricist view of knowledge-the assertion that knowledge is acquired by the gradual accretion of associations or stimulus-response connections that mirror the state of the environment. Indeed, Bower's conclusions point instead to the early importance of man's inherent structure, to the array of

species-typical readinesses to register upon and organise knowledge by highly ordered rules reflecting man's evolution.

Development goes forward by stages of organisation, each stage supplanted by a later stage when usable conflict occurs in the attempted application of earlier rules. It is the concept of usable conflict that distinguishes the author's view from that of conventional learning theory, for encounters with the environment are shown to be effective in producing change only when the infant is able to code the disparity between his expectancies and what actually transpires as a result of his responding. The codability of such mismatches depends upon the development of rule structures for interpretation the result of maturation shaped by experience.

For Bower, the initial structure of space for the infant is supramodal, a space into which the special senses are mapped. Sufficient experimental evidence is presented to raise sharp questions about both Berkeley's and Piaget's accounts of the associative or conceptual welding of the disparate spaces of eye, ear, and hand. New and searching techniques of research are now available—many of them invented by the author—that suggest that the hand knows what the eye knows before it has had its own experience of reaching under visual control. "It is highly improbable", he argues, "that any organism evolved a visual system or an auditory system that was not an outgrowth of the supramodal perceptual system . . . the system that responds to those properties of objects that can be specified via any sensory modality." In like vein, primitive visual objects, although they are elaborated by 'experience' simultaneously to encompass more properties, begin as inherently organised representations including more than a single modality. Witness the distressed surprise of the infant at the beginning of his manipulatory career who reaches out for a vintual object produced by polarised light and polarising spectacles, and finds no tactual feedback on grasping the target. An older child is less surprised, having come to differentiate palpability from visual object qualities.

Bower then argues that at the earliest stage, as indicated by studies of eyemovement and reaching, the infant has two disparate systems for identifying and conserving the identity of objects. One is for objects in motion and is based on a smooth pursuit system; the other is for static objects and is based on the saccadic system. Only with maturation and with appropriate experience, Bower argues, do infants develop rules for dealing simultaneously with the two systems.

As far as cognitive development is concerned, it is conceived of as the development of rules and regulatory processes that serve to organise different features of input and to sequence classes of behaviour rather than specific behaviours. Bower argues that no specific single behaviour is ever found to be crucial to development; adequate 'manipulatory' behaviour seems to develop even in limbless thalidomide children who carry out reaching and prehensive tasks with their mouths and teeth. "It is more parsimonious to assume change in an underlying concept which generates all of the behaviours, than to assume separate independent changes in each behaviour." Cognitive development, then, is seen as the progressive growth of increasingly comprehensive and differentiated control systems for constructing and sequencing behaviour. Bower presents a brilliant series of experiments to illustrate his point, centred upon the development of the concept of an object; how it is recognised as the same object though undergoing transformations in appearance, locus, and even singleness of identity.

But for all its virtues and power, this book is flawed in a way that destines it to criticism and challenge. It is flawed, ironically, by an overextension of its virtues; its very clarity of premise and argument, capped by uncompromising conclusions, makes it vulnerable. It will serve for years as a source of ideas for doctoral theses that will prove it inconclusive theoretically and wrong or partially wrong experimentally. For, in fact, there are not yet enough findings in the field of infant cognitive research to justify the inferential depth of Bower's arguments or the boldness of his conclusions. And though the experiments—perhaps too closely focused on work at Edinburgh, Harvard, and Geneva-are highly ingenious, and often brilliant, many of them are neither easily reproducible nor yet as conclusive as the author believes. (Witness his reliance on a doubtful study by Wertheimer indicating that newborns can orient visually to a voice source in space; or the still questionable reproducibility of some of his own results.) Indeed, because of the clarity and depth of the premises, the crisply unqualified statement of the findings, and the forthrightness (at times recklessness) of conclusions, the book will provide both a paradigm for next stages of research-and a whipping boy.

Yet, however much it will be attacked as 'premature', it is a major synthesis of work on the "original nature of mind" and will rank as one of the major books on human development to be written during the last decade.

Jerome Bruner

#### Not so elementary

Introduction to Particle Production in Hadron Physics. By S. Humble. Pp. viii+253. (Academic Press: London and New York, 1974.) £6.80; \$17.50.

THERE are some subjects that cannot be taught. One can only learn them by experiencing their harsh realities oneself. And one can only face such a task when driven by powerful motivation or attracted by the hint of great excitement to be found along the thorny path. Just such a subject is particle production in hadronic interactions.

That the author of this book has succeeded in producing a clear and balanced exposition of an incredibly difficult topic, is noteworthy. That he has failed to generate in us the necessary desire and excitement is a pity.

It is an unhappy, but surely not an unexpected fact, that as elementary particles are made to collide at ever higher energies, more and more debris of an increasingly complicated character is found to emerge from the collision. It is by studying this debris that we are supposed to be able to deduce the structure and the dynamical properties of the colliding particles. But what a daunting task. Even a description of the debris (that is, of the momenta and energies of the emerging particles) is too complicated to comprehend. How do you communicate to someone your findings concerning the dependence of a scattering amplitude upon the 14 variables needed to characterise a final state of 6 particles?

It is well nigh impossible, therefore, to hope to work back from data to dynamics. Rather, one postulates a dynamical model and attempts to confront it with the data.

There are several models currently in vogue, all interesting, none very convincing. The author attempts to describe and compare them fairly comprehensively. I found the discussion of dual models very informative, especially the treatment of their predictions and of the difficulties involved in their computation. The section on the analytical properties of production amplitudes is particularly valuable, if only for the fact that it exposes the enormous complexity of the problems encountered. Multiperipheral and multiregge models are presented with a nice balance between essential fact and gory detail.

The text is marred by ugly typography (even an equation split between pages) and by many errors. I cannot refrain from seizing on the following sentence which most appropriately appears just below a typographical error: "Hence, it must always be a good idea in a practical calculation to

derive as much of the formalism as one can for oneself, rather than relying on even the most detailed texts."

Other points to carp at: too little physical motivation for the ideas of 'scaling' in inclusive reactions; too much emphasis on the original work of Lurçat and Mazur on phase-space integrals, when more modern and simpler techniques exist; a lack of depth and a certain banality in summarising the properties of the models.

All said, however, this is a useful book. It is a good attempt to come to grips with an enormously difficult subject. But—and this must be stressed—it is essentially a book for the would-be professional, and it demands more than a modicum of expertise in the theory of elementary particles.

E. Leader



An early bird: the little owl, first introduced into Britain—where it is still relatively uncommon—during the last century. From Birds. By Christopher Perrins. Pp. 176. (Collins: London, September 1974.) £1.95. One of a series of books which also includes Woodlands, by William Condry, and Life on the Sea Shore, by John Barret.

## **Encompassing the North Atlantic**

The Ocean Basins and Margins. (Vol. 2, The North Atlantic.) Edited by A. E. M. Nairn. Pp. xiv+598. (Plenum Press: New York and London, 1974.) \$45.60.

THE North Atlantic must be among the most comprehensively surveyed ocean basins; and in order to arrive at a picture of its age and evolution it is necessary to consider not only the marine geophysical data amassed in recent years, but also a century or more of detailed geological research on the bordering continents. A few earth scientists are able to keep abreast of this vast volume of data. The rest of us, I imagine, tend to rely on the available reviews; and, ideally, these should be compiled in one authoritative and up-to-date volume covering every aspect of the problem. The fact that this second volume in the series The Ocean Basins and Margins comes close to realising the ideal should make it a much sought-after book.

Sins of omission are generally few, though chapters on the palaeomagnetic data from the North Atlantic continents and on sedimentation would have been welcome, and the northern reaches of the ocean deserve more attention. A general criticism which can be levelled at this volume, in its role as a source book, is that some chapters make reference to work published in 1973, whereas others, judging from their reference lists, were completed and on the editor's desk in 1971. There was surely ample time for the latter to be brought more up to date, if only by following the example given in M. J. Keen's chapter, which includes an appendix of useful references published after the completion of the manuscript.

On the credit side, however, the book does cover much the greater part of what needs to be known about the North Atlantic. The editors set the scene in their opening chapter with a qualified acceptance of the 1972 Pitman and Talwani model for the evolution of the ocean basin. Stehli and Keen review the geology of the well known western margin (Bahamas to Newfoundland) and point out some of the problems which remain to be solved. Owen (Western Approaches to the British Isles), Vigneaux (Biscay) and Dillon and Sougy cover the eastern margins, the last of these authors contributing a particularly valuable summary of western African geology. The major oceanic islands-Azores (Ridley, Watkins and MacFarlane), Canaries and Cape Verde (Dillon and Sougy) and Iceland (Noe-Nygaard) -are all described, the last in a good review of the North Atlantic Cainozoic volcanism. Evidence for an older 'proto-Atlantic' is to be found in the Lower Palaeozoic orogenic belts of the northern Appalachians (Williams, Kennedy and Neale), southern Caledonides (Dewey), and Scandinavia (Nicholson). The account of the tectonics of Newfoundland and the summary of the British Caledonides are particularly good. Additional references to the Caledonian orogeny, and much useful data pertaining to the early history of the ocean basin of the present northern North Atlantic can be found in a clear

account of the geology of Greenland's Atlantic coast. Finally, Fitch, Miller, Warrel and Williams bring together the literature on tectonic and radiometric age comparisons across the North Atlantic and Noltimier rounds off the volume with a comprehensive review of the geophysics of the ocean basin.

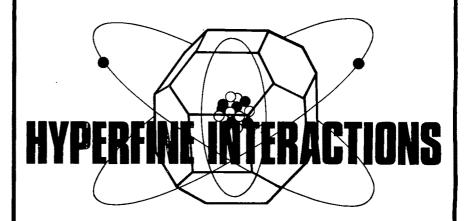
The book is well-produced, adequately illustrated, and there are extensive reference lists attached to each chapter. A subject index for the whole volume is provided, though a quick test of its usefulness revealed a "Silurian" entry which gives no page references for the Lower Palaeozoic orogens. The book contains the usual splattering of unimportant misprints, and one or two more serious gaffes, such as the ommission of the decimal points from the seismic wave velocities in Fig. 16 (p. 425). It is already somewhat dated, notably in relation to the marine geophysical and deep-sea drilling data published in the last couple of years. That is, however, inevitable and, with the reservations already mentioned, of no great significance. The important thing is that anyone now contemplating a study of the North Atlantic and its environs can have at his elbow one volume providing much of the necessary geological and geophysical background to his work. Every earth science library should have a R. J. Bailey copy of this book.

## Liquid scintillation counting

Applications of Liquid Scintillation Counting. By D. L. Horrocks. Pp. xiii+346. (Academic Press: New York and London, June 1974.) \$29.50; £14.15.

LIQUID scintillation counting originated 25 years ago when Ageno and his coworkers discovered that organic liquid solutions, when exposed to ionising radiation, emit scintillations which may be detected by a photomultiplier. Dr Horrocks' book provides an account of factors involved in internal scintillation counting. It also discusses applications of the technique, including chemiluminescence and bioluminescence, Cerenkov counting, pulse shape discrimination, flow-cell counting and large-volume counting. The discussion of quench correction unfortunately omits such topics as the relative quenching susceptibility of different scintillators, the different effects of impurity (chemical) and colour quenching, and the relative efficacy of the various quench correction methods. Nevertheless, the book can be recommended as a useful source of information on the applications of liquid scintillation counting. J. B. Birks

### announcing a new journal from North-Holland Publishing Co:



#### **Editors:**

H. DE WAARD, Physics Laboratory, University of Groningen, Groningen, The Netherlands B. DEUTCH, Institute of Physics, University of Aarhus, Aarhus, Denmark

#### Scope

The journal is concerned with research in the border regions of solid-state, atomic, and nuclear physics. Although the title and subject HYPER-FINE INTERACTIONS is one of the best examples of such a topic, other studies which utilize the modern techniques of atomic and nuclear physics may fall within the domain of the journal. The main concern is the understanding of border region physics, no matter what are the disguised variegated forms in which it appears.

The hyperfine interaction Hamiltonian is the logical basis for a large part of the subject matter to which this journal will be devoted because it contains products of atomic and nuclear quantities. In condensed matter, the Hamiltonian even spans solid-state physics. Hence such fields as: perturbed angular correlations, nuclear magnetic resonance, electric quadrupole resonance, beam-foil spectroscopy, nuclear orientation, and Mössbauer effect studies fall immediately within the journals domain. In addition, any atomic or solid-state studies via hyperfine spectroscopy (utilizing mesons, gamma rays, electrons, etc.) are within the scope. It can include problems related to solid, liquid and gaseous states as well as studies with biological material.

The title HYPERFINE INTERACTIONS is not meant to prevent contributions that do not directly involve such interactions from reaching the journal. For example, the journal is receptive to particle-beam studies which also explore the border physics region but

do not necessarily utilize hyperfine effects. Under this category lie channeling, nuclear lifetimes studied via channeling and implantation phenomena. In fact, studies which incorporate one of the disciplines of physics: solid-state, atomic or nuclear physics to study another would be generally acceptable.

#### Advisory editorial board

An editorial board is being formed and to-date the following scientists already accepted our invitation: B. Herskind, Copenhagen; E. Matthias, Berlin; J. A. Davies, Chalk River; N. Stone, Oxford; E. Bodenstedt, Bonn; G. Goldring, Rehovat; V. I. Goldanski, Moscow; T. Yamazaki, Tokyo;

#### Invitation to authors

The editors cordially invite colleagues to submit papers for publication within the scope of the journal. It should be realised that the refeering criteria will be high. Guidelines for preparing the manuscripts are available from the editors and publisher. Contributions should be sent to B. Deutsch, University of Aarhus.

#### Subscription information

The journal will be published in volumes of 6 issues; each issue will contain some 100 pages, first issue is scheduled to appear in spring 1975; In principle the journal will appear bimonthly. Subscription price: US\$42.50, Dfl.110.00 per volume, postage individed. Specimen copies will be supplied by the publisher.

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#### APPOINTMENTS VACANT

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PRINCETON UNIVERSITY
anticipates several openings for the 1975-76
academic year. One is at the Full Professor level
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to our ongoing programme in social psychology.
The other(s) at the Assistant Professor level are in
general experimental psychology, with concentration
in animal behaviour, developmental and comparative psychology, or personality research. These
latter anticipated openings are not rigidly tied to
any specific area of expertise. Sheer excellence in
research and teaching will override area-of-interest
considerations.

Nominations and applications should be sent to
the Chairman, Department of Psychology, Green
Hall, Princeton University, Princeton, New Jersey
08540. We are an equal opportunity employer with
a vigorous affirmative action programme. (2022)

#### ISLE OF MAN DIVING TECHNICIAN

DIVING TECHNICIAN

A Diving Technician is required at the University of Liverpool Marine Biological Station, Port Erin, for a period of two years to work on a project financed by the Wolfson Foundation to investigate the biological feasibility of farming scallops in cages in the sea. The work will involve much SCUBA diving and boat work. Applicants should hold at least a B.S.A.C. 3rd Class Diver qualification or equivalent. Some knowledge of biology might be advantageous but diving experience, a practical aptitude and enthusiasm are more important. Initial salary will be up to £1,860 per annum. Further particulars and application forms, which should be returned as soon as possible, may be obtained from the Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote Ref: RV/328/N. (2095)

#### FACULTY POSITION IN THEORETICAL ASTROPHYSICS AT CALTECH

The California Institute of Technology invites applications for the position of Assistant Professor of Theoretical Astrophysics. Applicants should have outstanding research abilities, as evidenced by publications and by strong recommendations from colleagues. We are particularly interested in applicants who will interact closely with our observational astronomers (radio, millimeter, infrared, optical, X-ray, and cosmic ray). However, we shall also consider outstanding applicants with research not tied closely to observations. Teaching ability will be taken into account in evaluating applicants.

research not tied closely to observations. Teaching ability will be taken into account in evaluating applicants.

Catech is an Equal Opportunity Employer with an Affirmative Action Program. We encourage applications from women and members of minority groups.

Letters of application should be sent to Maarten Schmidt, Executive Officer for Astronomy, California Institute of Technology, Pasadena, California 91125. Applications should include (1) a description of educational background; (2) a resume of research and teaching experience; (3) a list of all publications; (4) copies of all manuscripts submitted for publication but not yet published; (5) a statement of current research interests and future research plans; (6) a list of at least six astronomers or physicists who can evaluate the applicant's research, including at least three who know the applicant personally. The applicant should ask the latter three to send letters of evaluation directly to Professor Schmidt. All materials should reach Caltech before February 1, 1975. (2098)

#### **NEW HEBRIDES**

#### AGRICULTURAL EXTENSION **OFFICERS**

Required by the Department of Agriculture to take charge of an Extension Sub-District. The work is mainly concerned with New Hebrides producers and will involve touring among the islands by

Candidates should possess an Agricultural Degree or Diploma with at least 3 years good practical agricultural experience, preferably in the tropics. Some knowledge of French is necessary.

Appointment is on agreement for two to three years initially. Commencing salary according to experience in a scale approximately equivalent to £2,980 to £6,470 p.a., which includes an allowance, normally tax-free, in a scale £546 to £1,938. Benefits include gratuity of 25% of basic salary, free passages, generous leave, education allowance, children's holiday visit passages and rent free quarters. A car loan up to £600 and an appointment grant up to £300 may also be payable.

There is no income tax in the New Hebrides at present.

The post described is partly financed by Britain's programme of aid to the developing countries administered by the Ministry of Overseas Development.

For further particulars you should apply, giving brief details of experience to:

## crown agents

M Division, 4 Millbank, London SWIP 3JD, quoting reference number MID/740207/NF.

#### UNIVERSITY OF BRISTOL DEPARTMENT OF ANATOMY

The University invites applications for TWO LECTURESHIPS

in the Department of Anatomy. The posts are now vacant and may be taken up as soon as possible. For one of these appointments, preference will be given to candidates with an interest in developmental biology, and for the other, an interest in neurobiology. The Department is well-equipped for research in these areas. Candidates will be expected to take part in the research, teaching, and other activities of the Department. Salary will be within the range £2,118 to £4,896 or £3,462 to £4,896 (medically qualified) according to qualifications and experience. Applications, together with the names and addresses of two referees, should be sent not later than January 6, 1975 to the Secretary, Senate House, University of Bristol, Bristol BS8 1TH, from whom further particulars may be obtained (reference TLJ).

#### PAHLAVI UNIVERSITY DEPARTMENT OF BIOLOGY SHIRAZ, IRAN

SHIRAZ, IRAN

Applications are invited at the assistant/
associate professor's level for the following
positions on two year renewable contracts with an
annual salary range equivalent to U.S. \$12,000 to
U.S. \$16,000; depending on qualifications and
teaching experience.

Please send written applications, curriculum
vitae and confidential recommendations from
three referees to the Department of Biology,
Pahlavi University, Shiraz, Iran.

Applications are invited for the following fields:

1. Bacterial/Viral genetics
2. Developmental genetics
3. Cell organelle physiology
4. Plant Biochemistry
5. Animal physiology: osmoregulation

Plant Biochemistry •
Animal physiology: osmoregulation
Ornithology
Insect physiology
Plant growth regulators
Invertebrate zoology/protozoology
Terrestrial animal ecology/plant ecology
Echthology

Ichthyology Reproductive physiology Vertebrate neurophysiology

13. Vertebrate 14. Mycology

#### UNIVERSITY OF NEW SOUTH WALES

#### BIOCHEMIST/MICROBIOLOGIST/ MICROBIAL GENETICIST

(Lecturer—School of Biological Technology)

Technology)

The School practises a multi-disciplinary approach to Biological Technology and conducts research and teaching programmes in applied microbial genetics, microbiology, biochemistry (including enzyme technology) and biochemistry (including enzyme technology) and biochemistry and microbial genetics and considerable research experience in one, or preferably a combination, of the areas of study indicated. Successful applicant should have strong interests in projects of a potentially or directly applied nature. Duties will include formal undergraduate and postgraduate teaching and supervision of higher degree candidates. Appointment from February 1975.

Salary (under review): \$A9,002 to \$A12,352 per annum. Commencing salary according to qualifications and experience.

Details of appointment, including suberannuation, study leave and housing scheme, may be

Details of appointment, including sufferannuation, study leave and housing scheme, may be obtained from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H OFF.

Applications close in Australia and London on Janeary 17, 1975.

(2105)

#### UNIVERSITY OF LIVERPOOL RESEARCH ASSISTANT

RESEARCH ASSISTANT

Applications are invited from graduates with previous research experience particularly in biochemistry or a related discipline for a post of Research Assistant in the Department of Dental Sciences. The post to commence early in 1975 is financed by the Medical Research Council as part of a group studying the metabolism of sulphated glycoproteins of oral origin and is tenable in the first instance until October, 1976. Salary plus Threshold Supplementation and the option will be in the range £1,761 to £2,019 per annum of F.S.S.U. benefits.

Applications stating age, academic qualifications and experience together with the names of two referees should be received as soon as possible by the Registrar, The University, P.O. Box 147, Liverpool, L69 3BX. Quote ref. RV/323/N. (2111)

(2100)

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Required to head a mixed team of cytologists and chemists in work ranging from fundamental investigation into cell behaviour to the creation and evaluation of preparation techniques. A good understanding of cytology and organic chemistry is necessary together with the ability to organise a mixed discipline team working in collaboration with a computer based pattern recognition group. Many fields of R & D could have given candidates a suitable background but they must have enthusiasm, initiative and independence to advance this vital part of the Company's technology.

The Company's well equipped modern laboratories provide excellent working conditions and leisure facilities in pleasant rural surroundings ideally related to London and nearby Cambridge. Superannuation, sickness and life assurance schemes; B.U.P.A. group; tennis court; swimming pool; substantial re-settlement expenses, and generous salary. Contact the Technical Director for further information and an application form, quoting D/D79.

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(2123)

#### UNIVERSITY OF MELBOURNE CHAIRS IN BIOLOGICAL SCIENCES

The retirement of Professor Michael J. D. White F.R.S., F.A.A., at the end of 1975 and the appointment of Professor Geoffrey Burnstock F.A.A. to the Chair of Anatomy in University College London, will leave two chairs vacant in the biological sciences.

Applications for these chairs are invited from persons distinguished in research in any field of genetics or zoology or allied fields such as cell, developmental or molecular biology or animal ecology.

cell, developmental of indicedual viology of animal ecology.
Salary: SA19,614 per annum (under review).
Further information about the positions, including details of application procedure, superannuation, travel and removal expenses, housing assistance and conditions of appointment, is available from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, Londo WCIH 0PF.

Applications close on February 28, 1975.

#### THE UNIVERSITY COLLEGE OF WALES **ABERYSTWYTH** DEPARTMENT OF AGRICULTURE

A.R.C. RESEARCH ASSISTANT

required to work on aspects of winter cauliflower

production.

Applicants should have a good Honours degree in Horticulture, Agriculture or Agricultural Botany and the successful candidate will be required to assist with research on winter cauliflowers at the Department's Field Station at Trefloyne, Terphy

Salary from: £1,070 p.a. (plus dependants' allowance for those registering for a higher degree).

Further particulars and application forms may be obtained from the Registrar. Closing date: December 19, 1974. (2124)

#### UNIVERSITY OF BRISTOL DEPARTMENT OF CHILD HEALTH LABORATORY OF IMMUNOLOGY

Dr. Walter C. Henneberger, Chairman, Department of Physics and Astronomy, Southern Illinois University at Carbondale, Carbondale, Illinois 62901, U.S.A.

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LABORATORY OF IMMUNOLOGY

A POSTDOCTORAL BIOCHEMIST, preferably
with 2-3 years experience, is required in the
Laboratory of Immunology of the above department to purify and characterize the properties of
a new, and probably inherited, salivary substance
which is a serologically independent of the ABO,
Lewis and Sda blood group systems.
Commencing salary up to £2.412 per annum
plus Threshold supplements, depending on experience. The appointment will be funded by an
M.R.C. grant which runs for three years.
Applications, including curriculum vitae and
names and addresses of two referees, should be
sent to Professor N. R. Butler, Department of
Child Health, St. Michael's Hill, Bristol BSZ 8BJ.
(2132)

#### DALHOUSIE UNIVERSITY

Department of Pharmacology

Department of Pharmacology

Applications are invited for a POSTDOCTORAL FELLOWSHIP in one of the following fields:

(i) Cardiovascular of Pharmacology, (ii) Toxicology of Environmental Pollutants, (iii) Transport of neurotransmitters and electrolytes into presynaptic nerve terminals, (iv) Autonomic Pharmacology and Electrophysiology of Smooth Muscle.

Applicants, should not be more than 35 years of age, and should hold a Ph.D. degree with specialisation in one of the above mentioned areas from a recognised university. Initially this fellowship is granted for one year only, the annual stipend being \$9,100 (presently under revision) and subject to income tax. Travel grants may be provided to the fellow and spouse, if married.

Applications must be received no later than January 30, 1975, togither with a list of publications, curriculum vitae and at least two letters of recommendation to be sent directly from persons well acquainted with the applicant's personal and academic record to: Dr J. G. Aldous, Chairman, Department of Pharmacology, Sir Charles Tupper Medical Building Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4H7.

#### UNIVERSITY OF DURHAM DEPARTMENT OF PHYSICS

Applications are invited for the post of Post-doctoral Research Assistant from January 1, 1975 or as soon as possible thereafter. The successful candidate will be expected to pursue a research and development programme involving the application of neon flash tubes to nuclear particle detection with special reference to high energy gamma radiation. The programme will involve work at tion. The programme will in Science Research Council's involve v l's high

The appointment, which is funded by the Science Research Council, will be for a period of two

The salary will be on the scale from £2,055 to £2,793, plus threshold payments and F.S.S.U. benefits.

benefits.

Applications (3 copies) including the names and addresses of three referees should be sent by December 16, 1974 to the Registrar and Secretary, Science Laboratonies, South Road. Durham, DH1 3LE, from whom further particulars may be obtained. (2107)

#### UNIVERSITY OF LONDON INSTITUTE OF PSYCHIATRY POSTDOCTORAL NEUROCHEMIST

#### NEUROPHARMACOLOGIST

NEUROPHARMACOLOGIST

The appointment, which is for three years, is in the University Department of Neurology at the Institute of Psychiatry with an honorary contract at the M.R.C. Clinical Research Centre. Northwick Park, where some of the studies will be undertaken. The principal objectives are the study of the functions and inter-relations of folic acid and vitamin B12 in the nervous system including relationships to amine metabolism and the effect of anticonvulsant drugs. These studies will complement clinical investigations which are already in progress.

Starting salary within the range £2,311 to £3,144 including London Weighting according to qualifications and experience.

including London Weignung accounting fications and experience.

For application forms and further information please write to the Secretary, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London, SE5 8AF or telephone 01-703 5411 ext. 228.

(2112)

#### VISITS TO INDIA FOR YOUNGER SCIENTISTS AND SOCIAL **SCIENTISTS**

Applications are invited from suitably qualified British scientists, engineers and social scientists to visit India under the Younger Scientists Exchange Scheme. This Scheme is designed to promote mutual understanding and the formation of professional links between personnel in Indian and British universities and to facilitate the exchange of information and lides

versities and to facilitate the exchange of information and ideas.

Visits should preferably be of three months duration to enable a useful programme of work to be carried out, although in exceptional cases applications for shorter periods will receive consideration. Applicants should hold a staff appointment at a university, polytechnic or similar institution and preferably be aged between 27 and 35 years. Return tourist class air fares will be paid by the British Council and the Indian University Grants Commission will provide an honorarium to cover local expenses.

Grants Commission will provide an indicatual to cover local expenses.

Further information and application forms for wisits between late July 1975 and February 1976 may be obtained from the Director. Science Department, The British Council, 10 Spring Gardens, London SWIA 2BN. The closing date for applications will be January 31, 1975. (2135)

#### (PERSONNEL PLACEMENT, POSITION OPEN) VASCULAR-PLANT SYSTEMATIST HAWAII

Assistant or Associate Professor, Interests in tropical, Pacific and Asian plants; in a biosystematic and experimental approach to research; and in graduate and undergraduate instruction. Details available from Dr N. P. Kefford, Chairman, Department of Botany, 3190 Maile Way, University of Hawaii, Honolulu, Hawaii 96822. U.S.A. An Equal Opportunity/Assirmative Action Employer. (2122)

#### DEPARTMENTAL RESEARCH **ASSISTANT**

ASSISTANT

A Departmental Research Assistant is required to take charge of a Techniques Laboratory concerned with the preparation of targets and counters for Nuclear Physics experiments. Close collaboration with research staff and students using the Oxford accelerators is essential and some experience with Nuclear Physics apparatus and techniques is desirable, although not essential.

Applicants, who should be University graduates (preferably with Ph.D.), should write to Professor K. W. Allen, Nuclear Physics Laboratory, Keble Road, Oxford OX1 3RH. (2129)

#### UNIVERSITY OF THE WEST INDIES TRINIDAD

TRINIDAD

Applications are invited for the post of PRO-FESSOR OF AGRICULTURAL ECONOMICS in the Department of Agricultural Economics and Farm Management. Preference will be given to persons competent in quantitative economics and with broad training and experience in other aspects of Agricultural Economics. Duties will include teaching both undergraduate and postgraduate students and the supervision of research students Salary scale: TTS27,636 to TTS34,980 p.a. (£1 sterling=TT\$4.8). F.S.S.U. Unfurnished accommodation at rent of 10% of salary for maximum of three years; thereafter 20% of salary payable in lieu of housing. Family passages. Study and Travel Grants are also payable. Detailed applications (6 copies), including a curriculum vitae and naming 3 referees, should be sent by air mail, as soon as possible to the Secretary, University of the West Indies, St Augustine, Trinidad, Further particulars will be sent to all applicants. (2146)

#### UNIVERSITY OF BRISTOL SCHOOL OF CHEMISTRY POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited from Chemists or Physicists for an S.R.C. Postdoctoral Research Assistantship to work in the field of electronic laser resonance spectroscopy. The project will use a high resolution dye laser in studies of hyperfine structure, excited state dipole moments, and line widths of electronic spectra of polyatomic molecules

The appointment will be for one year in the first instance, with a posible extension to two years. Salary range up to £2,247 per annum plus Threshold supplements.

Applications with curriculum vitae and names of two referees to Professor R. N. Dixon, School of Chemistry, Bristol University, Bristol BS8 17S.

(2133)

#### UNIVERSITY OF ZAMBIA

UNIVERSITY OF ZAMBIA

Applications are invited for posts of (i) PROFESSOR/SENIOR LECTURER/LECTURER IN SCIENCE TEACHING METHODS and (ii) PROFESSOR/SENIOR LECTURER/LECTURER IN SCIENCE TEACHING METHODS in the School of Education. Applicants should possess a relevant first degree, a postgraduate teaching qualification and higher degree as well as experience in secondary school teaching. Preference will be given to those with teacher training experience in a frican countries. Salary scales: Professor K7,400 to K7,800 p.a. Senior Lecturer K5,600 to K6,600 p.a. Lecturer K4,000 to K5,400 p.a. (£1 sterling=K1.49). The British Government may supplement salaries of all married appointees in range £516 to £1,152 p.a. (sterling) or supplement salaries of single appointees to the levels of Senior Lecturer and Professor in range £78 to £204 p.a. (sterling) and provide children's education allowances and holiday visit passages. This supplementation is unlikely to be provided to single appointees to Lecturer level. Family passages; various allowances; superannuation and medical aid schemes; regular overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 refereees, should be sent by airmail, not later than January 6, 1975 to the Registrar, University of Zambia, P.O. Box 2379; Lusaka, Zambia, Applicants resident in U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London WIP 0DT. Further particulars may be obtained from either address.

## **Glaxo**

## Technical Officer-Fermentation Development ULVERSTON, CUMBRIA

We have a vacancy for a Technical Officer, who should either have an HNC or a first degree qualification. A sound knowledge of Analytical Chemistry is essential. Candidates must have initiative and should be versatile: any experience in Microbiology, Automatic Instrumentation. Electronics or Control Systems would be advantageous.

The Fermentation Development Department is essentially interdisciplinary and conducts investigations in Microbiology, Genetics, Biochemistry, Fermentation and Biochemical Engineering in support of the production of antibiotics, Vitamin B12 and Enzymes. The Analytical Section provides a range of service facilities and initiates Analytical development including novel approaches to automation and the continuous monitoring and control of fermentations.

The salary will be according to age and experience.

The Company offers good conditions of service including bonus, pension and sick pay schemes. Ulverston is a pleasant market town situated on the southern fringe of the Lake District National Park.

Please apply in writing to:



The Personnel Officer (JE), Glaxo Laboratories Limited, North Lonsdale Road, Ulverston, Cumbria, LA12 9DR.

(2128)

#### UNIVERSITY OF CAMBRIDGE DEPARTMENT OF ENGINEERING

#### RESEARCH ASSISTANT IN EXPERIMENTAL AERODYNAMICS

The Head of the Engineering Department hopes to appoint a research assistant to conduct experimental investigations into problems connected with aircraft engine components. The post is supported by industry at a salary up to £3,636 p.a. plus threshold, depending on qualifications. Qualified applicants will have an honours degree in Engineering or Physics, Applications, together with curriculum vitae and the name of a referee, should be sent to the Secretary, Faculty of Engineering, Trumpington Street, Cambridge CB2 1PZ, not later than January 1, 1975. (2134)

#### THE UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY DEPARTMENT OF CHEMISTRY EXPERIMENTAL OFFICER (Ref: CH/108/R/AI)

(Ref: CH/108/R/AI)

Applications are invited for the post of Experimental Officer in the Department of Chemistry to be responsible for the operation of an Electron Spin Resonance Spectrometer, and to participate actively in the teaching and research programmes with which it is concerned.

The post will be particularly attractive to candidates who can combine an interest in and practical knowledge of electronics with a high level of organic and inorganic chemical ability including research and development experience and interest in reactive intermediates and mechanistic aspects of chemistry. in reactive intermediates and of chemistry.

Salary within the scale: £1,758 to £3,114 with

F.S.S.U. Requests for application forms to the Registrar, U.M.I.S.T., Sackville Street, Manchester M60 1QD, to be returned not later than January 18, 1975. (2140)

#### UNIVERSITY OF THE WEST INDIES **JAMAICA**

Applications are invited for (a) LECTURESHIP (b) ASSISTANT LECTURESHIP IN THE DEPARTMENT OF ZOOLOGY, tenable as soon DEPARTMENT OF ZOOLOGY, tenable as soon as possible. Preference will be given to persons with an interest in marine ecology Salary scales: (a) J\$6,168 to J\$9,768 p.a. (b) J\$5,006 to J\$5,486 p.a. (£1 sterling=J\$2,11). F\$S,U Study and Travel Grant. Unfurnished accommodation at rent of 10% of salary for maximum of three years, thereafter 20% of salary payable in lieu of housing Family passages. Detailed applications (6 copies), including a curriculum vitae and naming 3 geferees, should be sent by air mail, as soon as possible to should be sent by air mail, as soon as possible to the Registrar, University of the West Indies, Kingston 7, Jamaica. Detailed particulars are avail-able and should be obtained from the same source before an application is made.

#### DEPARTMENT OF ANATOMY UNIVERSITY OF CAMBRIDGE

UNIVERSITY OF CAMBRIDGE.

Applications are invited for a postdoctoral research associate position to work on lymphocyte trapping at the placental focto-maternal interface during pregnancy; this work is part of a general project involving investigation of immunological relationships in pregnancy, and the application of immunological techniques to developmental studies. Applicants should preferably have experience in handling and separation of sub-populations of lymphocytes; some experience in embryology would be helpful but is not essential. The post is available for one year in the first instance, with a starting salary of approx. £2,400, and starting on January 1, 1975 or as soon as possible thereafter. Applicants should send details to Dr. M. H. Johnson, Department of Anatomy. Downing Street, Cambridge CB2 3DY, and include a full curriculum vitae, publications, the names of two referees etc. as soon as possible. (2150)

## CHROMATOGRAPHY IN DRUG RESEARCH

The role of Central Research's Analytical Chemistry department is to devise and develop methods to control potential new drugs from their discovery to marketing. A principal aim is the generation of technical data for submission to world-wide regulatory agencies.

To maintain the technical support currently required we need an additional chemist to work as a chromawe need an additional chemist to work as a chromatography specialist, supervising the activities of two members of staff. The range of duties is varied and includes the application of GLC, HPLC and TLC to a wide variety of problems including homogeneity assessment, the quantitative control of impurities and stability studies. The job involves collaborating with a large number of research staff, and provides an excellent opportunity to gain a wide experience of separation science in a pharmaceutical research environment.

The successful applicant will probably have had about ten years post-qualification (BSc., G.R.I.C.) experience, be used to supervising and training junior staff and command a starting salary of c. £3,500.

Salaries are reviewed annually, and a bonus scheme in operation. Other terms of employment include pension and death benefit schemes, flexible working hours, and considerable financial assistance with removal expenses.

Our laboratories are situated in a pleasant coastal/ rural area at Sandwich, Kent.

Applications, giving brief details of age and experience should be addressed to:



D. W. Sells. Personnel Manager, Central Research, Pfizer Limited. Sandwich, Kent.

(2137)

#### RESEARCH ASSISTANT

RESEARCH ASSISTANT

Applications invited for the post of research assistant to study the mechanisms of resistance and susceptibility of wheat to rust fungi. The work envisaged is at the biochemical level and is supported by the Agricultural Research Council.

Applicants should have had at least three years post-graduate research experience in Botany, Biochemistry or Microbiology but not necessarily Plant Pathology, although some knowledge of this subject could be an advantage. Salary within range £2,118 to £2,412 p.a. plus F.S.S.U. if appropriate, £213 London Allowance (under review) and Threshold payments.

Application by December 27 with a curricultum vitae and the names of two referees to Dr R. N. Strange, Department of Botany and Microbiology. (N) University College London, Gower Street, London WC1E 6BT. (2149)

#### RESEARCH ASSISTANT

• RESEARCH ASSISIANI
required for studies of radiation effects on an experimental chondrosarcoma in the Department of Physics. Graduate in biological subject preferred with good Honours degree. Successful candidate may register for a higher degree. Salary £1,698 p.a. plus 2 by £79 and threshold allowances. Applications, with names of two referees, to Dr N. F. Kember, Dept. of Physics, The Medical College of St. Bartholomew's Hospital, Charterhouse Square, London, ECIM 6BQ, quoting reference 688. (2138)

#### **ELECTRONICS TECHNICIAN** (Grade 5)

required for small research group in Zoology and Comparative Physiology Department, investigating ultrasonic communication and echolocation systems of animals. Responsibilities will include maintenance of various advanced instruments and the development of new equipment and techniques for investigating ultrasound in air. The appointment is temporary for one year in the first instance. Salary scale £2,439 to £2,895 p.a. plus £228 p.a. London Weighting. Five weeks in total annual leave. Five day week. Letters only to Assistant Secretary (Establishment) ET/Z, Queen Mary College, Mile End Road, E1 4NS, stating age, qualifications and experience. (2113)

#### UNIVERSITY OF LEEDS

DEPARTMENT OF **EXPERIMENTAL** PATHOLOGY AND CANCER RESEARCH

Applications are invited for the post of POSTDOCTORAL RESEARCH FELLOW to work in collaboration with Dr R. C. Garner on the development of short-term tests for chemical carcinogens.

Candidates should have a background in tissue culture techniques and would be expected to carry out research on nuclear changes induced by carcinogenic and non-carcinogenic chemicals in mammalian cells.

The appointment, which is sponsored by a Medical Research Council grant, will be for up to three years within the salary range of £2.118 to £2.757 plus F.S.S.U. depending on age and experience, and commencing on January 1, 1975 or as soon as possible thereafter.

Applications in writing, giving full details of career etc. together with the names of two academic referees should be sent by December 18 to the Registrar, The University of Leeds, Leeds LS2 9JT quoting reference 89/1. (2092)

#### UNIVERSITY OF READING GRADUATE

#### RESEARCH ASSISTANT

required in Department of Zoology, from January I to join a small team studying the ecology of chalk streams. The post involves field and laboratory work. Starting salary in range £1,500 to £2,000 p. a. Apply to Dr J. F. Wright, Department of Zoology, University of Reading, Whiteknights, Reading RG6 2AJ, from whom further constitutions are provided by the control of particulars are available, (Ref: MN 58). (2164)

#### UNIVERSITY OF SYDNEY LECTURER IN AGRONOMY (Crop Nutrition)

A lecturer is required in the general field of the mineral nutrition of crops. Applicants should hold a first degree in science or agricultural science, and a Ph.D. or equivalent research interests and experience in the discipline. The Department has 15 academic positions and by mid-1975 will be housed in new laboratories and offices. In addition there are excellent facilities for teaching and research at the Department's Field Stations (Camden, New South Wales).

The position advertised is permanent but may

The position advertised is permanent but may be filled for three years in the first instance with possibility of renewal after that date, or in certain cases return fares.

Salary range: \$A9,002 to \$A12,352 p.a.

Saiary range: 5A9,002 to \$A12,352 p.a. Applications, including curriculum vitae, list of publications and 'names of three referees, by January 6, 1975, to the Acting Registrar, University of Sydney, New South Wales 2006, Australia, from whom further information is available. Information also available from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H OPF. (2156)

#### CHELSEA COLLEGE UNIVERSITY OF LONDON

Applications are invited for the post of Laboratory

#### **TECHNICIAN** (Grade 4)

in the Department of Physiology of the Basic Medical Sciences Group. We are looking for someone with appropriate qualifications and experience. The successful candidate should have experience of electrophysiological and medical instrumentation and be willing to make his contribution to experimental classes and research in the Department, Salary Scale: £2,475 to £2,856 (including £228 London Weighting, which is under review)

#### UNIVERSITY OF ST. ANDREWS

Botanic Garden: Department of Botany

Botanic Garden: Department of Botany
Applications are invited for the post of
Records Officer/Taxonomist, who will be
responsible to the Curator for the maintenance of the records and the identification
of plants in the Botanic Garden. A qualification in Botany or Horticulture with taxonomic interests is required. Appointee may be
registered for a research degree in the
Botany Department. Salary scale £1,860 to
£2,187. Further information may be obtained from the Curator, University Botanic
Garden, St. Andrews, KY16 8RT. Applications, stating qualifications and experience
and giving the name of a referce, should
be submitted to the Establishments Officer,
University of St. Andrews, College Gate,
St. Andrews, Fife, KY16 9AJ, by December
16, 1974. (2119)

#### MEMORIAL UNIVERSITY OF NEWFOUNDLAND

HEAD OF THE DEPARTMENT OF GEOLOGY

The Memorial University Geology Department has 18 faculty members, a full technical and secretarial staff, 25 graduate students, and about 60 undergraduate majors. In 1975, it will occupy 40,000 square feet in a new building, which will contain a range of teaching and research laboratories and equipment (flume tank, XRF, XRD, microprobe, etc.).

Recent research in the December 18

microprobe, etc.).

Recent research in the Department has been dominantly within the context of Appalachian and Labrador studies, but there has lately been a broadening of approach, especially in marine and sedimentary geology.

No specific field of research is required for the new Department Head, but the suitable candidate must be a highly competent scientist with an ability to understand and encourage a wide range of geological research, much involving close cooperation with other University departments, government and industry. Enquiries should be addressed to Dr D H. Rendell, Associate Dean of Science, Memorial University of Newfoundland, St. John's, Newfoundland, Canada, AIC, 557.

Applications should be received by January 31,

Applications should be received by January 31, 1975. (2157)

#### UNITED KINGDOM ATOMIC ENERGY AUTHORITY

#### INFORMATION **OFFICER**

(HIGHER SCIENTIFIC OFFICER)

Applications are invited for the post of Information Officer in the Library and In-formation Department of the Reactor Group Headquarters, Risley, Warrington.

The duties of the post involve informa-tion searches in a wide range of technical, scientific and commercial literature; monitor-ing journals for references for a weekly bulletin and subject-indexing of specialised reports.

The work requires tireless curiosity and a desire to help engineers, scientists and managers by tracking down information for them in the literature.

The successful candidate will have the use of two large modern libraries of reports and published literature, staffed and managed by qualified personnel.

#### QUALIFICATIONS:

Experience of information work is desirable. In addition, candidates should have a degree, HNC or HND in a scientific, mathematical or engineering subject, or equivalent qualifications in library and information work.

£2,635 on a scale rising to a maximum of £3,610 per annum plus "threshold" pay agreement which, at present, provides a supplement of £13.92 a month.

Please write for application form, quoting reference S608A/J.14 to Staff Officer, Room E303, United Kingdom Atomic Energy Authority, Reactor Group Headquarters, Risley, Warrington, WA3 6AT.

Closing date for the return of application forms: December 24 1974. (2094)



#### UNIVERSITY OF DUBLIN Trinity College

#### CHAIR OF MICROBIOLOGY

Applications are invited for appointment to this Chair, which will fall vacant on July 1, 1975.

Further particulars may be obtained from:

The Secretary to the College West Theatre Trinity College Dublin 2.

Formal applications should, if possible, reach the Secretary (2061)before January 6, 1975.

#### CENTRAL & SOUTHERN HEALTH DISTRICT (Teaching) LIVERPOOL A.H.A. (T)

#### BASIC GRADE BIOCHEMIST

required at

DEPARTMENT OF CHEMICAL **PATHOLOGY** ASHTON STREET, LIVERPOOL 3

Salary scale £1,689 rising to a maximum of £2,994 per annum.

Application form and job description obtainable from the District Administrator, 80 Rodney Street, Liverpool, L1 9AP, to be returned by December 27, 1974. (2131)

#### THE UNIVERSITY OF **MANCHESTER**

#### RESEARCH ASSISTANT IN OCCUPATIONAL HEALTH

Applications invited from mechanical or aeronautical engineers, or candidates qualified in fluid mechanics, to study the air flow in the alveoli of the human lung. Opportunities exist to register for a higher degree. Salary according to qualifications and experience. Post tenable for up to 3 years. Further particulars and application forms (returnable by December 20) from the Registrar, The University, Manchester, M13 9PL. Quote ref: 245/74/N. (2160)

#### THE CITY UNIVERSITY

**Department of Chemistry** 

#### POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited from suitably qualified candidates in materials science or electrochemistry to work on an S.R.C. sponsored project on Semiconducting Oxide Anodes,

The starting salary will be on the scale £2,118 to £2,580 per annum plus £213 London Allowance and threshold payments of £229.68 per annum.

Please apply to Dr A. C. C. Tseung, Department of Chemistry, The City University, St. John Street, London, (2143)

#### "METALS ABSTRACTS"

The international abstracting service for metallurgy offers permanent positions as SENIOR EDITORIAL ASSISTANTS The work consists of editing and checking abstracts for publication. A science degree, preferably in metallurgy, physics, or chemistry, is necessary and a working knowledge of a foreign language would be an advantage.

Applications, stating age, education, qualifications, and experience, to Dr T. Graff, Metals Abstracts, The Metals Society, 1 Carlton House Terrace, London SWIY 5DB

(2148)

#### WELSH NATIONAL SCHOOL OF MEDICINE RESEARCH ASSISTANT

required in the Department of Dermatology required in the Department of Dermatology to work on the physical effects of topical applications on the epidermis and stratum corneum. This post is financed by Vick International and is suitable for a graduate in the biological sciences; it is a two-year appointment in the first instance which may be renewable thereafter. Salary on the scale £1,689 to £2,562, depending on qualifications and experience.

Applications should take the form of a brief curriculum vitae with the names and addresses of two referees and should be sent to the Registrar, Welsh National School of Medicine, Heath Park, Cardiff, CF4 4XN quoting reference no. M6/29.

Further details may be obtained from Dr R. Marks, Senior Lecturer in Dermatology.
Department of Medicine, Welsh National
School of Medicine, Heath Park, Cardiff. (2162)

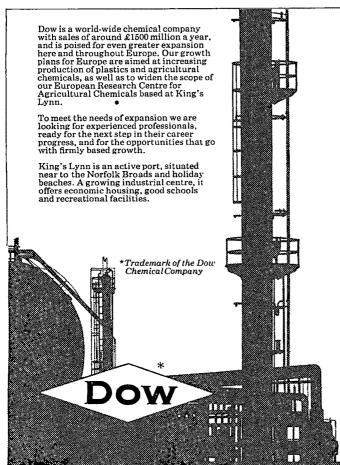
#### UNIVERSITY COLLEGE **GALWAY**

#### SENIOR TECHNICIAN

Applications are invited for the post of senior technician in the Department of Microbiology from persons holding the Advanced Certificate of City and Guilds, A.I.M.L.T., H.N.C., Diploma of Medical Laboratory Sciences, Technicians Diploma of the Department of Education or equivalent.

Applications giving details of qualifications, experience and the names of two referees to the Professor, Department of Microbiology, University College, Galway.

(2151)



### Registration/ Toxicology Assistant

to join our recently formed and rapidly expanding Agricultural Research Division at King's Lynn. The Assistant, male or female, will prepare submissions for product registration, maintain central registration and toxicological files, and liaise with contract research laboratories and field scientific personnel throughout Europe, the Middle East, and Africa. This is an important appointment demanding a graduate with a good degree in Biological Sciences, Veterinary Surgery, Pharmacology or Pharmacy.

Post-graduate experience should preferably have been concerned with the development of agricultural pesticides and/ or animal health products especially with government registration. Fluency in written and spoken English is obviously essential, and a knowledge of other European languages, especially German and Italian, would be an

An attractive starting salary will be paid and benefits include contributory pension scheme, BUPA membership and comprehensive relocation allowance. Career development prospects are excellent within the European Division.

Please write for an application form, quoting reference U.194. to:

Geoffrey J. Webb, Personnel Manager, Dow Chemical Company Limited, King's Lynn, Norfolk.

(2142)

#### ZOOLOGICAL SOCIETY OF LONDON

NUFFIELD INSTITUTE OF COMPARATIVE MEDICINE

#### LIPID BIOCHEMIST

with postdoctoral experience for two year experimental study on foetal and neonate brain development. Salary up to £2,970 according to experience; F.S.S.U. benefits. Curriculum vitae to Assistant Establishment Officer, Zoological Society of London. Regent's Park, London, NW1 4RY.

#### UNIVERSITY OF GLASGOW RESEARCH ASSISTANT

Applications are invited for the post of research assistant in the department of Cell Biology to study the nitrate reductase of free-living and symbiotic root nodule bacteria. Minimum qualifications for the post are a degree, or the equivalent, together with some experience of biochemical and/or microbiological techniques.

The work is supported by the Agricultural Research Council and the appointment is for two years in the first instance. Salary will depend on qualifications and experience but is likely to be over £2,000 plus F.S.S.U. The post is available from December 30, 1974.

Applications, including a curriculum vitae and the names and addresses of two referees, should be sent as soon as possible to Dr R. M. Daniel, Department of Cell Biology, University of Glasgow, Glasgow G11 6NU.

In reply please quote Ref. No. 3595M (2163)

#### UNIVERSITY OF SYDNEY LECTURESHIP IN MICROBIOLOGY

The appointee will assist in teaching Microbiology courses, supervise student research projects and undertake research in conjunction with one of the research groups studying: Microbial treatment and recycling of industrial wastes including pollution controls of water ways; bioassay and degradation of herbicides and pesticides, all aspects of biological nitrogen fixation.

pects of biological nitrogen fixation.

Candidates should have good knowledge of the whole field of basic Microbiology and be able to teach in most areas of Microbiology to Science, Agriculture, Marine Science and Pharmacy students. A higher degree or equivalent with teaching and research experience, will be given preference. Administrative and industrial experience will also be taken into consideration.

The position advertised is permanent and may be filled for three years in the first instance with possibility of permanency after that time, or in certain cases return faires.

Salary range: \$A9,002 to \$A12,352 p.a.

Salary range: \$A9,002 to \$A12,352 p.a. Enquiries to Professor Y. T. Tchan in the

Applications including curriculum vitae, list of publications and names of three referees, by January 15, 1975, to the Acting Registrar, University of Sydney, New South Wales, 2006, Australia. Information about conditions of appointment and application procedure available from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF, (2155)

#### ROYAL POSTGRADUATE MEDICAL SCHOOL STUDENT/JUNIOR TECHNICIAN

required in the Immunoassay Laboratory of Department of Medicine for work involving radioimmunoassays of thyroid hormones in various endocrine disorders. Minimum qualifications. GCE "O" level passes in English. Mathematics and 2 Science subjects. Salary on scale £1,107 to £1,848 per annum plus threshold payment.

Applications to the Personnel Officer (743 2030 Ext 93), R.P.M.S., Hammersmith Hasnital, DuCane Road, London W12 0HS, quoting ref. no. 2/490, (2170)

#### THE POLYTECHNIC OF NORTH LONDON

Holloway Road, London, N7 8DB

#### Research Assistantship in Genetics

Applications are invited from graduates with good honours degrees for the post of Research Assistant, to work under the super-vision of Dr C. R. Bantock on the cyto-genetics or population genetics of gastropods. The successful applicant will be expected to register for a higher degree.

Salary scale: £1,544 by £55 to £1,654.

Apply in writing, giving qualifications, experience and the names of two referees to the Head of the Department of Biology & Geology, The Polytechnic of North London, Holloway Road, N7 8DB.

#### THE COUNCIL FOR SCIENCE AND SOCIETY

invites applications for the post of ASSISTANT SECRETARY, organising Working Parties and Conferences. A knowledge of some branch of science, technology or medicine would be useful, but not essential. Particulars are available from The Council for Science and Society, 3/4 St. Andrew's Hill, London EC4V 5BY. (2169)

#### UNIVERSITY OF SUSSEX

SCHOOL OF BIOLOGICAL SCIENCES

#### TEMPORARY LECTURESHIP IN **GENETICS**

A temporary Lectureship in Genetics for one year, with possible extension for a second year, is immediately available.

Teaching responsibilities will be primarily for a second year class, and will cover aspects of Metazoan Genetics.

the research interests of the associated group is in the developmental genetics of Drosophilia, including Embryology and cell culture, and good facilities are available. Candidates with other research interests will also be considered. Appointment will be made on the Lecturer scale £2,118 to £4,896 per annum plus threshold payments, as soon as possible. The research interests of the associated

Application forms may be obtained from the Secretary of Science, Science Office (E), University of Sussex, Falmer, Brighton BNI 9RH. (2165)

#### UNIVERSITY OF THE WITWATERSRAND

Johannesburg, South Africa DEPARTMENT OF GEOLOGY

Applications are invited for the following posts:-SENIOR LECTURESHIP IN PETROLOGY

Applicants should have substantial research experience in the field of igneous and/or metamorphic petrology, a familiarity with experimental petrologic techniques, and a Ph.D. or equivalent qualification.

petrologic techniques, and a Ph.D. or equivalent qualification.

SENIOR LECTURESHIP IN ENGINEERING GEOLOGY AND GEOTECHNICS

Applicants should state clearly their experience in Application of geomathematics to the study of the properties of geological materials.

Starting salaries for these posts will be determined according to qualifications and experience within the following ranges:

Senior Lecturer: R8,460 to R11,250 (annual increments not less than R360); Lecturer: R6,300 to R9,180 (annual increments R360).

R1.00=£0.62=\$US1.44.

Intending applicants may obtain the information circular relating to these posts, and outlining the attractive fringe benefits, from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa. Applications giving full career and porsonal details, should be submitted not later than January 30, 1975. UK applicants may obtain the information sheet relating to this post from the London Representative. University of the Witwatersrand, 278 High Holborn, London W.C.1.

#### **HEAD: DIVISION OF** VIRAL PRODUCTS

NATIONAL INSTITUTE FOR BIOLOGICAL STANDARDS AND CONTROL

Applications are invited for the post of Head of the Division of Viral products in the above Institute. The primary object of the Institute is the control, in relation to the Medicines Act 1968, of biological substances used in human medicine. The Division, which at present has a scientific staff of twelve, is responsible for the control and standardisation of viral products, activities which are well supported by research work. Salary would be according to age, qualifications and experience within the range of £6,105 to £8,121.

The Institute at present is one of the establishments of the Medical Research Council, but it is expected that, as a result of impending legislation, the management will at some time in the near future be transferred to a new employing authority, viz. the National Biological Standards

Applications should be sent to the Director, National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3 6RB by the end of January 1975, and should include the applicant's curriculum vitae, list of publications and the names of three referees. (2121)

### information scientist

#### crop protection research

Have you a degree in chemistry or a biological science?

Could you accept responsibility for all the information needs of one of our research areas-research chemicals, biological data, patents, other literature, competitors' products etc?

Can you communicate effectively with technical people at all levels and in several disciplines?

Can you work on your own initiative with enthusiasm and determination?

Could you take advantage of and help improve our computerised information services?

Are you aged 23-30?

If you fit these requirements and have preferably had some experience of information work in and R & D environment, we would like to hear from you.

Attractive salary and fringe benefits include Profit sharing Scheme and Pension Fund. Assistance with house purchase may be applicable.

lealott's Hill Research Station is situated on a pleasant farm estate between Bracknell and Maidenhead.

If you are interested in this job, please write or telephone for an application form to:

Mr S. R. Stephenson, Personnel Officer, ICI Plant Protection Ltd., Jealott's Hill Research Station, Bracknell, Berks. Tel: Bracknell 24701

(2144)



ST. MARY'S HOSPITAL, MANCHESTER

#### RESEARCH ASSOCIATE IN HUMAN **IMMUNOGENETICS**

We would like to receive applications from individuals interested in human immunogenetics with special reference to leukagmia. Using recent technical developments and mixed lymphocyte culture we are studying normal and tumour associated antigens. The successful candidate should have a good honours degree, an M.S.C. in immunology or related subject, or be a graduate interested in entering the field of immunogenetics. post is annually renewable for a maximum of two and a half years from January 1, 1975 and will offer a suitable candidate modern facilities and participation in the Department's future developments. Salary according to Whitley Council Scale for N.H.S. Scientific Officer. Send curriculum vitae and names of two referees by December 16, 1974 to Dr R. Harris, Department of Medical Genetics, St. Mary's Hospital, Hathersage Road, Manchester M13 0JH.

#### Department of the Environment Princes Risborough Laboratory

### nformation Scientist

■ In Application Services Section concerned with disseminating results of research ■ Organise courses, seminars, symposia and visits ■ Arrange presentation of Laboratory's work at exhibitions ■ Liaise with TV and film producers and develop the use of Video Recording

Equipment as visual aid.

Degree/HNC or equivalent in science or engineering 
Knowledge of

timber technology an advantage  $\square$  Age under 27  $\square$  Appointment as Scientific Officer (over £1800-£2900)  $\square$  Ref: SA/80/HA.  $\square$  Application forms (to be returned by 7 January 1975), from Establishment Office, Princes Risborough Laboratory, Aylesbury, Bucks HP17 9PX.

Ministry of Agriculture, Fisheries and Food Pest Infestation Control Laboratory, Tolworth

## **Zoologist/Ecologist**

■ In Rodent Research Department ■ Develop and improve methods of field testing rat poisons and new control techniques 
Conduct field trials.

☐ 1st/2nd hons degree or equivalent in Zoology or Ecology ☐ Knowledge of statistics desirable ☐ Age under 30 ☐ Appointment as Higher Scientific Officer (£2950-over £3850) or Scientific Officer (over £2050-£3150), according to age and experience [ ] Ref: SB/12/AF.

☐ Application forms (for return by 6 January 1975), from Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke 29222 ext. 500 (or, for 24 hour answering service, London 01-839 1992).



(2130)

#### FELLOWSHIPS AND STUDENTSHIPS

#### THE UNIVERSITY OF SHEFFIELD DEPARTMENT OF CHEMISTRY

DEPARTMENT OF CHEMISTRY

Applications are invited for an S.R.C. (C.A.S.E.)

STUDENTSHIP for a project involving the synthesis of a novel group of heterocyclic compounds. These are designed to possess controlled chemical reactivity and potential herbicidal character. The work will be carried out in conjunction with I.C.I. Plant Protection Limited and part of the programme will be performed at their Jealott's Hill Research Station. Applicants should have (or expect to obtain this summer) a good Honours degree or G.R.I.C. in chemistry and the successful candidate will be expected to register for a Ph.D. Applications including a curriculum vitae and names and addresses of two referces to: Dr G. M. Blackburn, Department of Chemistry, The University, Sheffield S3 7HF (from whom further information is available). Quote Ref. R. 153/G.

#### UNIVERSITY OF ABERDEEN UNIT FOR RESEARCH ON ADDICTIVE DRUGS

#### POSTDOCTORAL FELLOWSHIP

Applications are invited for above post supported by the Medical Research Council. Appointment is for two years with possible extension for third year. Salary in range £2,247 to £2,580 plus threshold payments.

Applicants who hold a Ph.D. in Organic Chemistry or Biochemistry are expected to take part in work in progress in the Unit concerned with the identification and synthesis of the endogenous compound with morphine-like actions recently discovered in the central nervous system. Facilities will be provided in the Unit and in the Department of Chemistry.

Further particulars from the Secretary, The University, Aberdeen with whom applications (2 copies) should be lodged by December 22, 1974.

(2096)

## POSTDOCTORAL FELLOWSHIPS IN ASTRONOMY AND ASTROPHYSICS AT CALTECH

ASTRONOMY AND ASTROPHYSICS AT CALTECH

The California Institute of Technology invites applications for Postdoctoral fellowships in astronomy and astrophysics. Awards, beginning in summer or autumn 1975, will normally be for one year with likely renewal for a second year. Caltech is active in observational astronomy (radio, millimeter, infrared, optical, X-ray, and cosmic ray; solar, planetary, interplanetary, stellar, interstellar, and extragalactic), laboratory astrophysics (nuclear, atomic, and chemical), and theoretical astrophysics (with emphasis on cosmology, galaxies, stars, coherent emission processes, and nuclear and relativestic astrophysics). It is expected that postdoctoral fellowships will be available in the fields of (1) radio astronomy, (2) X-ray astronomy, (3) cosmic ray physics, (4) solar physics, (5) lunar science, (6) planetary science, (7) nuclear astrophysics, and (9) theoretical astrophysics.

Research capabilities include: the radio facilities of the Owens Valley Radio Observatory including conventional and VLB interferometry and spectrometry; new millimeter-wave antennas now under design and construction; the optical and infrared facilities of the Hale Observatory; rocket-borne X-ray telescopes; airplane-borne infrared telescopes; balloon- and satellite-borne cosmic-ray telescopes; balloon- and satellite-borne cosmic-ray telescopes; accelerators and facil-ties for measuring nuclear and agomic reaction rates and cross sections; laboratories for isotopic analyses of meteorites and lunar rocks; an IBM 370/158 computer; a PDP-10 computer; and a terminal into a CDC-7600 computer; and a terminal into a CDC-7600 computer.

Caltech is an Equal Opportunity Employer with an Affirmative Action Program. We encourage applications from women and members of minority

applications from women and members of minority groups.

Applicants should write for application forms to Professor Maarten Schmidt. Executive Officer for Astronomy, California Institute of Technology, Pasadena, California 91125. All application materials should reach the California Institute before February 15, 1975. (2099)

#### UNIVERSITY OF YORK DEPARTMENT OF BIOLOGY

DEPARTMENT OF BIOLOGY

A Research Studentship funded by the Multiple Sclerosis Society is available immediately for work which can lead to a higher degree, on the association and interaction between different cell compartments and the myelin sheath on central nerve tissue in health and disease. Applicants should have a good first degree in Biochemistry, Physiology or a Biological Science and applications together with the names and addresses of two referees (or request for further information) should be sent to Dr M. G. Rumsby, Department of Biology, University of York, York YO1 5DD, as soon as possible.

(2115)

#### **NEWCASTLE-UPON-TYNE** WILFRED HALL FELLOWSHIP

Applications are invited for the Wilfred Hall Fellowship (tenable for two years from October 1, 1975) from persons who have shown themselves able to carry out original research in the field of Pure Science. The value of the Fellowship is £2,118 in the first year and £2,247 in the second

year.
Further particulars and application forms (which must be returned by January 15, 1975) may be obtained from the Registrar, University of Newcasife upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne, NEI 7RU. (2125)

#### UNIVERSITY OF SUSSEX RESEARCH FELLOW IN SCHOOL OF **BIOLOGICAL SCIENCES**

Applications are invited for a postdoctoral research fellowship involving studies of electrophysiological and bicchemical changes in the membrane of Acetabularia during cap regeneration. Training in electrophysiology is required, preferably with some biochemical experience as well. The appointment, supported by the S.R.C., is in the Development Biology Group under Dr Goodwin and is tenable for three years from October 1, 1974 or three years from October 1, 1974 or thereafter with a salary on the scale £2,118 to £2,412, plus F.S.S.U. benefits and threshold payments.

Applications with names of two referees should be sent as soon as possible to Dr B. C. Goodwin, School

of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG. (2117)

#### UNIVERSITY OF EDINBURGH RESEARCH FELLOW/ASSOCIATE IN CLINICAL PHARMACOLOGY

Applications are invited from graduate chemists, biochemists, pharmacists or pharmacologists for the above post in the University Department of Therapeutics, The Royal Infirmary, Edinburgh. The successful applicant will be working with clinical pharmacologists on studies of drug metabolism in relation to toxicity, and experience in an sufficient chemistry is desirable.

The post is supported by a grant and is tenable for a maximum period of three years. Salary on the scale £2.118 to £3.285, according to qualifications and experience.

the scale 12.18 to 13.725, according to qualifica-tions and experience.

Applications, giving the names of two referees, should be sent to Dr. L. F. Prescott, University Department of Therapeutics, The Royal Infirmary, Edinburgh EH3 9YW, not later than December 16, 1974. Please quote reference 5054. (2118)

#### UNIVERSITY OF SOUTHAMPTON POSTDOCTORAL RESEARCH **FELLOWSHIP**

FELLOWSHIP

Applications are invited for an S.R.C. Postdoctoral Fellowship in Inorganic Chemistry to investigate the reactions of ligands coordinated to
metals of the platinum and adjacent groups.
Applicants should have had experience in synthetic
inorganic chemistry but synthetic organic chemists
will also be considered.

The appointment will be tenable for one year
and may commence at any time between January
and October 1975. The salary will be in the range
£2,118 to £2,247 per annum (plus threshold payments) and will include F.S.S.U. benefits. Applications, giving curriculum vitae, the names of two
referees, a list of publications and reprints (if
available) should be addressed to Dr George
Rouschias, Department of Chemistry, The University, Southampton SO9 5NH. (2126)

## ICI Postdoctoral Research Fellowships

Applications are invited under the terms of the ICI scheme for the award of postdoctoral research Fellowships. Awards for the academic year 1975/6 will be the last made under this scheme, which will end with the completion in 1977 of projects started in October 1975. Fellowships may be held at the universities listed below. Additionally, up to five of the Fellowships may be held at appropriate European research centres.

University of Aberdeen University of Aston in Birmingham University of Bath The Queen's University of Belfast University of Birmingham University of Bradford University of Bristol **Brunel University** University of Cambridge The City University Cranfield Institute of Technology University of Dublin, Trinity College University of Dundee University of Durham University of East Anglia University of Edinburgh University of Essex University of Exeter University of Glasgow

Heriot-Watt University University of Hull The National University of Ireland (Dublin, Cork, Galway) University of Keele University of Kent at Canterbury University of Lancaster University of Leeds University of Leicester University of Liverpool University of London Loughborough University of Technology University of Manchester (including University of Manchester Institute of Science and Technology) University of Newcastle upon Tyne University of Nottingham

The Open University University of Oxford University of Reading University of St. Andrews University of Salford University of Sheffield University of Southampton University of Stirling University of Strathclyde University of Surrey University of Sussex University of Ulster University of Wales (Aberystwyth, Bangor, Cardiff, Swansea, University of Wales Institute of Science and Technology) University of Warwick University of York

The Fellowships, tenable for two years, can be held in any field relevant to ICI's own research interests: this includes many branches of chemistry, physics, the biological sciences, applied mathematics, engineering, and technology. For the guidance of the universities and intending candidates an exemplary list of research topics is available. Candidates should not be more than 28 years of age on taking up their awards, and must hold the Ph.D. degree or have equivalent research experience. Successful candidates will be expected to commence their Fellowships on 1st October 1975.

The stipend will depend upon age and experience but will generally be within the range £2,100-£2,700 per annum (subject to review), together with FSSU benefits. In the case of Fellows working at a European research centre an appropriate adjustment will, if necessary, be made to cover differences in the cost of living, and a contribution will be made towards travelling expenses.

Forms of application may be obtained from the Registrar/Secretary of the university at which the candidate wishes to pursue his

research (except in the case of London University, where enquiries should be directed to the Academic Registrar), and must be returned duly completed by 30th January, 1975. Candidates desiring to work in Europe (and only those candidates) should apply to Dr. D. C. Moore, Research and Development Department (Academic Liaison), Thames House North, Millbank, London SW1P 4QG. Candidates need apply to only one university, but may apply to not more than three if they so wish. In the case of multiple applications, separate forms must be completed in respect of each university. Among other information, candidates will be required to submit a brief curriculum vitae, a clear summary of the research programme proposed, and the names of two referees. Other things being equal, preference will be given to candidates proposing to carry out their research at institutions other than that from which they make application.

The final choice of candidates will be made by a National Selection Committee, comprising representatives of the universities and of ICI

## Research **Fellowships**

at Continental Universities Applications are invited for awards by the CIBA-GEIGY Fellowship Trust for the academic year 1975-76.

Two classes of award will be made as follows:-

CIBA-GEIGY Fellowships

CIBA-GEIGY Fellowships are offered to graduates of universities of the United Kingdom and the Republic of Ireland for research into chemistry, biochemistry, chemical technology including chemical engineering, and biology, particularly in its relation to chemistry, at an agreed university including a technological university on the continent of Europe.

Qualifications recent first degree university graduates normally resident in the UK who intend to undergo training in research for a doctorate or other higher degree at an agreed European university including a European technological university.

Duration of the award the minimum required by the university regulations subject to satisfactory progress, with the possibility of an extension for an additional year.

Scale of awards £1,900 per annum (£2,200 per annum if married) plus travelling expenses.

The candidate should not be in receipt of any other award.

Senior CIBA-GEIGY Fellowships

Senior CIBA-GEIGY Fellowships are offered to lecturers, senior lecturers, readers or those holding comparable positions at United Kingdom or Republic of Ireland universities who are also graduates of these universities and wish to undertake special study at an agreed continental European university including a European technological university.

Duration of the award minimum period of six months and up to two years.

Scale of awards £5,000 per annum plus travelling

Further details and application forms, which should be submitted before 31 January 1975, can be obtained from The Secretary, The CIBA-GEIGY Fellowship Trust, 30 Buckingham Gate, London SW1E 6LH.

(2110)

#### UNIVERSITY OF WARWICK POSTDOCTORAL RESEARCH FELLOW IN MOLECULAR **BEAM SCATTERING**

Applications are invited for an S.R.C. Post-doctoral Research Fellowship in the Department of Molecular Sciences to work on the investigation of four centre ion-molecule reactions. This will include experimental work using an existing crossed molecular beam apparatus at the Thorngon Research Centre and the calculation of potential energy surfaces and scattering calculations. The applicant should have experience in high vacuum work and preferably in molecular beams. The post is available immediately and will be for one year in the first instance with the possibility of renewal. Starting salary will be in the range £2,118 to £2,580 p.a. plus threshold, with F.S.S.U. benefits. Application forms and further particulars may be obtained from the Academic Registrar, University of Warwick, Coventry CV4 7AL, to whom applications should be sent as soon as possible. Please quote Ref. No: 17/A/74.

#### POSTDOCTORAL FELLOW

required for work on interaction of carcinogens with nucleic acids, starting early 1975 for 1-2 years. Stipendium approx. 1450 DM net per

Applications to Dr P. Kleihues, Max-Planck-Institut für Hirnforschung, 5 Cologne 91, W. (2153)

#### UNIVERSITY OF LEEDS

#### DEPARTMENT OF GENETICS

Applications are invited for a Research Studentship in the Department of Genetics. The successful applicant will be required to undertake studies on detection of heterozygotes, and on prenatal diagnosis, in Duchenne muscular dystrophy using isoenzymes of creatine phosphokinase and potassium conductance of cells.

Please apply to Dr M. Crawford, Department of Genetics, University of Leeds, Leeds LS2 9JT, by December 16, 1974. (2139)

#### UNIVERSITY OF ABERDEEN POSTDOCTORAL RESEARCH FELLOWSHIP IN BIOCHEMISTRY

Applications are invited for above post to determine the primary structure of phospho-glycerate mutase and other glycolytic enzymes. The project is supported by the M.R.C. and is for a period of up to three years. The post is a collaborative one with the X-ray crystallography group at the University of Bristol who have determined the tertiary structure of the mutase at 3.5A resolution. Salary within range £2,118 to £2.412 per annum (plus threshold payments).

Further particulars from the Secretary, The University, Aberdeen with whom applications (2 copies) should be lodged as soon as possible

#### PORTSMOUTH AND KINGSTON **POLYTECHNICS**

DEPARTMENT OF GEOLOGY

Applications are invited from graduates, for TWO Ministry of Overseas Development Studentships (£890 p.a.), one at each institution, starting immediately, for a period of three years, to carry out Ph.D. research as part of an investigation into the geology and mineral resources of the igneous ring-complexes of Sudan under the Direction of Dr J. R. Vail, Head of Geology Department, Portsmouth Polytechnic and Dr D. C. Almond, Senior Lecturer, Kingston Polytechnic.

Two periods in Sudan will be involved so the ability to carry out independent field work in the desert is essential. A knowledge of Arabic and ability to drive would be use-

Applications to and further particulars from Dr J. R. Vail, Department of Geology, Portsmouth Polytechnic, Burnaby Road, Portsmouth, PO1 3QL by January 6, 1975. (2161)

#### AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for appointment to the following:

JOHN CURTIN SCHOOL OF MEDICAL RESEARCH

#### RESEARCH FELLOW DEPARTMENT OF BIOCHEMISTRY

The Research Fellow will collaborate with an existing research group in the Department in work in one of the following areas: Protein chemistry, with particular reference to the isolation and characterisation of membrane proteins from normal and mutant bacteria; Biochemical genetics of bacterial oxidative phosphorylation; Enzyme reactions mechanisms with particular reference to allosteric enzymes.

Further details of the research work may be obtained from Professor F. Gibson, John Curtin School of Medical Research, Australian National University, Canberra

Closing date: March 1, 1975.

#### RESEARCH SCHOOL OF PHYSICAL SCIENCES

#### FELLOW/SENIOR RESEARCH FELLOW/RESEARCH FELLOW IN COLLOID SCIENCE, BIOPHYSICS

Appointment would be in the Department of Applied Mathematics, Institute of Advanced Studies, which works in Colloid and Interface Science with a bias towards membrane biophysics (Professor B. W. Ninham) and in Vision Research and Optical Communications (Dr A. W. Snyder), It has close working links with the Department of Neurobiology (Professor G. A. Horridge) and the Department of Theoretical Physics and works also in Liquid State Physics. Enquiries are especially invited from people interested in the Colloid field and/or Polymer Science. The Department would particularly welcome a person with some knowledge of or an interest in membrane biology, and it is expected that some opportunity to do experimental work may arise if desirable. Closing date: January 31, 1975.

Closing date: January 31, 1975.

SALARIES (under review): Salary on appointment will be in accordance with qualifications and experience within the ranges: Fellow \$A10,771 to \$A14,704 p.a.; Senior Research Fellow \$A13,163 to \$A15,548 p.a.; Research Fellow \$A9,002 to \$A12,269 p.a.; current exchange rates are approximately \$A1: 56NP: \$US 1.31.

OTHER CONDITIONS—Tenure: Fellow for five years in the first instance with possibility of extension to retiring age; Senior Research Fellow and Research Fellow normally for three years in the first instance with possibility of extension to a maximum of five years.

Reasonable travel expenses are paid and assistance with housing is given for an appointee from outside Canberra. Superannuation is on the F.S.S.U. pattern with supplementary benefits.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should write to the Association of Commonwealth Universities (Applis.) 36 Gordon Square, London WC1H 0PF, for further particulars before applying. (2154)

#### THE ROYAL SOCIETY WARREN RESEARCH FUND COMMITTEE

The Warren Research Fund Committee of the Royal Society invites applications for a WARREN RESEARCH FELLOWSHIP. In accordance with the terms of the Trust, this appointment may be for scientific research in metallurgy, engineering, physics or chemistry or for the use or application of such research or its results in or for industry and industrial development.

The Fellowship will be tenable in any university, industrial research laboratory or research institution or at any other place in the United Kingdom subject to the approval of the Committee. Candidates should supply the usual personal details and two referees. Testimonials will not be considered. The subject of the proposed research, and the place at which it would be carried out, together with the name of the head of the department, whose consent should first be obtained, must be stated.

The appointment is tenable for four years in the first instance from October 1, 1975 and may be renewed for further periods at the discretion of the Committee. The scale of stipend will be either £5,097 by £180 to £5,817 per annum or £3,870 by £177 to £4,578 per annum, both with threshold payments in addition and appropriate superannuation benefits.

Applications should be made on forms to be obtained from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London, SW1Y 5AG, and should be received not later than February 14 1975. (2116)

#### THE UNIVERSITY OF LEEDS

#### Department of Biochemistry

Applications are invited for a POST-DOCTORAL RESEARCH FELLOWSHIP for the study of muscle membranes in dystrophic animals. This work will involve the purification and analysis of the components present in these membranes. Preference will be given to biochemists or chemists with some experience in protein chemistry and/or membrane biochemistry.

The appointment will be for one year in the first instance, renewable for a further two years, and is financed by a grant from two years, and is financed by a grant from the Muscular Dystrophy Group of Great Britain. Salary, according to age and ex-perience, will be in the range of £2,118 to £2,757 plus F.S.S.U. Threshold supplements are additional to this scale. The appointment can be made as soon as convenient.

Enquiries or applications (with curriculum vitae and the names of two referees) should be sent as soon as possible to Dr C. F. Louis, Department of Biochemistry; 9 Hyde Terrace, Leeds LS2 9LS (Tel. 0532 36171).

(2120)

#### LECTURES AND COURSES

THE FIFTH SUMMER COURSE IN TISSUE CULTURE

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Department of Anatomy, University of Saskatchewan, Saskatoon, Sask. S7N 0W0, Canada.
Telephone: 306-343 2661. (2167)

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The course begins in September 1975, and runs for one calendar year. Closing date for applications, January 15. Particulars and application forms from Registrar, University College London, Gower St, WCIE 6BT. (2168)

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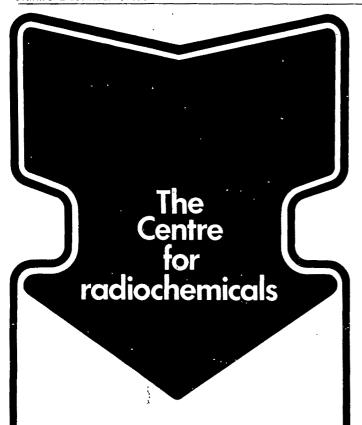
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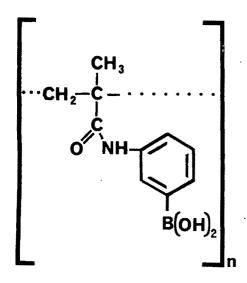
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#### Guide to authors

- Articles are up to 3,000 words in length with at most six displayed items (figures and tables) and may either be reports of major research developments in a subject or broader reviews of progress.
- Letters are brief reports of research of unusual and wide interest, not in general longer than 1,000 words; at most they have three or four displayed items (figures and tables).
- 'Matters Arising' permits occasional short discussion of papers that have previously appeared in *Nature*. There is a limit of 300 words.

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Nature for December 20 will be a double issue, and the next issue after that will appear on January 3 1975.



### Paying the piper

THE perks of working in the Nature office are not extensive. No doubt if the journal were dedicated to the evaluation of stereo equipment or gourmet meals or travel to the tropics we would not lack material benefits, but reporting on advances in science and on science policy hardly leads to obvious free handouts. Can you imagine the Science Research Council's report being distributed with a modest fusion reactor or an 'autonomous house' to keep the press happy? Of course we get the occasional lunch, the odd trip abroad and even an executive jet flight now and then to help us see things in an objective light. We also get an agreeable pile of diaries and calendars (and if Ciba-Geigy read this I'd like them to know how I've enjoyed their calendar of fully dressed high class girls and how I hope I'll get another one this Christmas). And the other day a tiny crate containing a bottle of champagne to help me join in celebrating the launching of a brand new film on embryology the makers of which I can't plug in these columns. But the most interesting perk I've yet had came a few evenings ago.

Bayer AG Leverkusen makes drugs and like every other large company these days a million and one other things also. It also has a symphony orchestra conducted by Rainer Koch and comprised entirely of its own employees. That is unusual, you may say, but not unique; companies keep all sorts of things besides football teams these days. Aren't there famous bands such as Black Dyke Mills Band and Foden Motor Works Band? (I can remember advertisements in the local paper, "Stanton Iron Works requires a tenor euphonium player; office job will be found"). What's new about the Bayer Philharmonic?

I cannot claim to be a music critic, although I do reckon to know a fair bit about orchestras, and not through regurgitating comments from record magazines. Nevertheless it was clear that the Bayer Philharmonic would have stood comparison with the best of amateur orchestras anywhere in the world. For its concert in the Fairfield Hall, Croydon, the company had invited along staff, customers, business associates and, no doubt, rivals ("got anything like that in your company, eh?"). The orchestra started with Vaughan Williams's "The Explorer", film music of the 1950s and a rarity indeed in English concert programmes. Nice as it was as a gesture it didn't really come off. Vaughan' Williams is so English and has to be appreciated in the context of green fields, sheep and country pubs; there is something intangibly incongruous in eighty German drug company employees in dinner jackets trying to make the folk songs sing. But enough chauvinism; there was little to fault elsewhere in the programme.

Dinorah Varsi joined the orchestra to perform Chopin's somewhat over-inflated piano concerto in E minor. The orchestra was more in its element here and

it was only the occasional ability all amateur orchestras have to make the piano sound slightly out of tune which detracted from a fine performance. The orchestra concluded the evening with an excellent rendering of Brahms's First Symphony, in which the only weakness of substance was the inability of the woodwind, particularly clarinet and oboe, to make much impression over the top of a fine body of strings.

Now what is the moral of all this, for moral there has to be, even in a Christmas issue? Not that it must take a great deal of 'spare time' to drill so precise a band. Not even that you should go out and buy Bayer products to keep the orchestra in business. Rather that it is a much more intelligent form of patronage of the arts than most companies indulge in at present.

Sponsorship and patronage is under much discussion, because in a period of financial gloom the first thing a business does is economise on its peripherals. There is an obvious tendency to regard sponsorship as advertising and thus to seek to attach the company's name to the best known events both in art and sport. In the short run this probably does little harm and a fair bit of good in keeping admission prices down. But in the long run anything that tends to centralise and to emphasise the cult of the excellent at the expense of personal participation is bad. It is good to know that various London symphony orchestras, theatres and operas are being supported by big business but it would be better to know that big business was equally among the grassroots, raising the levels of competence and enthusiasm of amateurs and young professionals in Scunthorpe, Swansea and Kilmarnock. With modest help, perhaps most intelligently supplemented by the provision of professionals to coach or even write for them, such groups can clearly flourish, and what's more young professionals can be encouraged. It may even have a longer lasting effect on the company image than a once-a-year mention in the programme of a London performance.



AFTER the Franco-Prussian war of 1870-71, it is well known that in many districts in France a new vegetation sprang up, evidently the result of the invasion. It was believed that this vegetation would become acclimatised. It is not so, however, L'Institut informs us; at least very few of the species introduced in this way appear likely to continue to flourish on French soil. In the departments of Loiret and Loir-et-Cher, of 163 German species, the half at least have already disappeared, and the surviving species diminish in vigour each year. Scarcely five or six species would appear to manifest any tendency to become acclimatised; these are, according to M. Nouel, Alyssum incanum, Trifolium resufinatum, Kapistrum rugosum, Melilotus sulcata, and Vulpia ligustica. On the plateau of Bellevue, where in 1871 many strange species were seen, M. Bureau has been able to find only one-Trifolum resupinatum. M. Gaudefroy also, who in 1871 and 1872 found many adventitious plants, has been able to collect only two this year-Ranunculus macrophyllus and Linum angustifelium.

From Nature, 11, 135, December 17, 1874.



Sahel drought: photo by Nick Fogden

Looking for a strategy to feed the World over the next decade, the World Food Conference put together a comprehensive action pack—but not all its elements looked easily compatible and the conference called on science and research workers to bridge the gap. Robin Sharp who has attended numerous United Nations conferences as a journalist and now heads Oxfam's Public Affairs Unit was in Rome writing for PAN, the highly successful conference newspaper.

To imagine that giant leaps for mankind could spring directly from a two-week 'talkfest' like the World Food Conference would be to misconstrue the nature and purpose of such events. Indeed it is probably better that the United States, the EEC and the OPEC countries did not come forward at that moment with the billions of dollars being asked for them, because with so much stress laid on the easy-to-grasp, short term crisis, we might all have returned home in a dangerous

state of euphoria. The temptation to call the conference a success would have risked implying that somehow it had 'solved' the problem of how the world is to feed itself in the next 10 years. And nothing could be further from the truth.

The principal function of the Rome meeting was to act as a means of harnessing all available political energy and then channelling it out to power the productive machinery of government, international agencies, research institutes and so on. This it has done and, as far as can be judged at such close range, done to measurably good effect. A momentum has been created; the job now will be to use and sustain it.

Already the dimensions of the immediate crisis are more widely known—some accomplishment in itself. The people of Africa, Asia and Latin \*America have spent about \$10,000 million in the past year to buy abroad the food they need to live. Now the money has run out, but at the end of the conference they were still short of 7.5 million tons of grain, which would cost about \$2,500 million at market prices. Over the next mine months, reckoned by average

grain consumption in the developing countries, we are talking about the food of 45 million people. And without some drastic intervention, the story over the next 10 years will be the same, with a high rate of compound interest.

Faced with disaster warnings of such magnitude, a world food strategy based on large scale, capital-intensive technological inputs was virtually irresistible: the answers had to be on the same scale as the problems. The potentially serious flaw in this approach is that it depends on how the problems are defined-and they get defined in their 'macro' aspect because that is the only way they are manageable. In fact, the 30-man Food and Agriculture Organisation (FAO) task force which drafted the conference's Action Plan had originally thought of submitting alternative strategies-one based on high technology, the other on labourintensive schemes utilising intermediate technology and fed in much closer to the grass roots. In the end they tried to blend the two together but the result was something of an oil and water mixture, with a preponderant dose of oil.

To improve the strategy's consistency. Third World delegates and others introduced many amendments underscoring their conviction that the aims of increased food production must be fitted into a pattern of integrated rural development, taking full account of social, cultural and environmental conditions. And perhaps it was because the massive macro schemes often seemed incompatible with the needs of the millions of poor rural workers that the final conference document laid special stress on the need for more research.

In a lengthy resolution from its First Committee, the conference expressed "concern at the inadequate amount of basic and particularly applied research directed to developing new agricultural technology suited to the needs of developing countries, as well as weaknesses in adaptive research, training and extension to achieve more effective transfer and utilisation of both existing and new technology . . ." There is a paucity of trained technical personnel, the resolution added, and much current research "lacks coordination and makes inadequate use of important information already available from research in ecologically comparable regions."

Among its nine proposals on research issues, the conference recommended:

- Rapid establishment of a global network of plant genetic resource centres and the extension of this to animal gentic resources.
- Intensified research by national and international institutions on the application of meteorological information to land-use and other agricultural planning, such as the development of alternative cropping strategies to suit different weather conditions.
- That the FAO/World Bank Consultative Group on International Agricultural Research (CGIAR) should study the feasibility of an international programme on the use of remote sensing techniques in agriculture, including the use of data from the Earth resources satellites.
- That extensive adaptive research programmes be developed, involving testing in farmers' fields the economic and technical viability of new technology and thereby tailoring recommendations to suit specific locations, farming situations and socio-economic conditions.
- That means be found to give the developing countries better access to research equipment and activities, including germ plasm resources.

On specific areas of research, the Action Plan pointed out that the socalled Green Revolution has so far only made a substantial impact on varieties of wheat, maize and rice. It suggested that barley and triticale—a hybrid between triticum (wheat) and secale cereals (rye) species-were other grains deserving a greater research effort, triticale in particular because of its potentially high yield, high protein content and potential for flourishing on marginal lands. Also, the plan stated, no work is at present supported at international level on oil seeds such as sunflower, safflower and rapeseed, although their oils are important in the diets of many developing countries. Similarly more work is justified on root crops such as cassava, which provide the energy requirements of perhaps 40 million people.

While accepting the need of extended research and development in these fields, less attention was given to the equal need for high-yield varieties able to survive without sophisticated inputs. For it is the cost and scarcity of these essential inputs—chemical fertiliser, diesel fuel for irrigation pumping, petrol for transport-which has suddenly made the first Green Revolution turn sour for many who committed their livelihood to it. And this, in turn, leads to wider questions which the World Food Conference made no attempt to assess. How far has the present crisis been exacerbated by existing large scale rural development projects? How much capital has gone into underused facilities which could have been used to create jobs and therefore food-buying power?

While pondering these dilemmas, there are formidable problems of a more practical kind to be dealt with. For example:

Development of land and water resources. The secretariat's programme

Botswana food programme; FAO photo



called for the improvement of 46 million hectares of existing irrigated lands, the expansion by 1985 of a further 23 million hectares under irrigated agriculture and development of 153 million hectares of new land in rainfed areas. The cost• of this programme would, however, amount to \$89,000 million, including about \$30,000 million in foreign exchange—and even before the conference began several delegations had registered their scepticism as to whether resource transfers on this scale could be realistically envisaged or fully absorbed.

Post-harvest losses. The importance of improved food storage received only a passing mention in the final resolutions but figures cited in the Action Plan illustrate the need for better facilities at all levels of distribution Postharvest losses in cereals, as a result of mildews and fungus diseases, rodents and insects, are estimated at 5 to 10% but up to 40% in exceptional cases. Losses of 30 to 40%, are common in perishable fruit and vegetables. Insects consume the most nutritious part of the grain, leading to deterioration in its calorific, protein and vitamin contents; and damaged grain absorbs moisture, encouraging the growth of harmful micro-organisms.

Nutrition. Information on food consumption patterns and on their consequences for the nutrition and health status for the majority of people in developing countries is insufficient, said the 15-joint resolution on this subject. Improved knowledge about how to prevent malnutrition through better use of available food resources, including human milk, is essential. The conference called on governments to explore the "desirability and feasibility of meeting nutrient deficiencies, through fortification of staples or other widely consumed foods, with amino acids, protein concentrates, vitamins and minerals." It also recommended the establishment of a global nutrition surveillance system to monitor, inta alia, the condition of disadvantaged groups, and an inventory by the FAO of vegetable food resources other than cereals, including roots, tubers, legumes and "also those from unconventional sources."

Now these and the many other recommendations of the Rome conference are in the hands of the United Nations, its various specialised agencies and individual governments, waiting to be implemented.

Ultimately, as the conference Secretary-General, Mr Sayed Marei, phrased it, "judgment on the success or failure of this conference is going to be made by hungry men and women in Africa or Asia, or by a hungry child..." But how long will they have to wait?

T HE REPORT of the work of Dr J. W. King and his colleagues at the Appleton Laboratory (252, 2; 1974) recalls articles contributed to *Nature* almost a century ago by William Stanley Jevons, economist and statistician, in which he sought to evidence the relation between sunspots and commercial crises (19, 33; 1878 and 19, 588; 1879).

Jevons's early education was in mathematics, chemistry and botany. He later developed an interest in, and wrote widely on, meteorology. This scientific training influenced his approach to the study of economics. In Letters and Journal of W. Stanley Jevons, edited by his wife (Macmillan, London, 1886) we read:

'He published in Waugh's Australian Almanac for 1859, "Some Data concerning the Climate of Australia and New Zealand", a paper over fifty pages in length, which is best described by his closing words: "My object has been to present in an available form such accurate numerical data as are attainable, and secondly, to group together general information as to the winds, rains, rivers, floods, the geographical features of the country, and the meteorological circumstances of this part of the globe, so as to show what remarkable problems have to be solved, and what interesting connections of cause and effect may ultimately be traced and proved.""

The significance of his method was that, translated into the context of 'political economy', it represented a new approach to the investigation of economic phenomena. In a paper read to the meeting of the British Association at Cambridge in 1862, "On the Study of Periodic Commercial Fluctuations, with five diagrams", Jevons wrote:

"It seems necessary, then, that all commercial fluctuations should be investigated according to the same scientific methods with which we are familiar in other complicated sciences, suche especially as meteorology and terrestrial magnetism."

In 1862, when this was written, Jevons was concerned with seasonal changes and not with the longer swings of the trade cycle; and his interest in meteorology had not led him to the theory of solar variation to explain these larger swings. His famous conclusions on the underlying causes of the trade cycle were advanced in two papers presented to the British Association in 1875 and 1878.

The fate of the first paper, "The Solar Period and the Price of Corn" is recounted in J. M. Keynes's Essays in Biography (Mercury Books, London, 1961):

'Thorold Rogers' "History of Agriculture and Prices in England", which

began to appear in 1866, provided Jevons with material for analysing wheat prices over a long period. The commercial crises in his own lifetime had occurred at intervals of ten or eleven years: 1825, 1836-39, 1847, 1857, 1866. Might there not be a connection between these things? "I am aware," Jevons concluded, "that speculations of this kind may seem somewhat far-fetched and finely-wrought; but financial collapses have recurred with such approach to regularity in the last fifty years, that either this or some other explanation is needed," Nevertheless he soon repented of publishing

## Sunspots and the business cycle

J. R. Sparkes, lecturer in economics at the University of Bradford, looks at the work of W. S. Jevons (below), who tried to relate sunspot activity and commercial crises a hundred years



what was no better than a bright idea. "Subsequent enquiry convinced me that my figures would not support the conclusion I derived from them, and I withdrew the paper from publication"."

But Jevons still did not regard the enquiry as worthless and encouraged to discover, after withdrawing his paper, that Sir William Herschel had tried as early as 1801, although with negative results, to explain the variation in the price of corn by the decennial variation of sunspots. Thus by 1877 he was working at the subject again and was more convinced than ever that there was some connection between sunspots and the price of corn. In a letter to Professor J. d'Aulnis de Bourouill in February 1878 he wrote, concerning the years when there were commercial crises, "The periodicity is remarkable, and the average length of the period is somewhere about 10.3 years, so nearly the same as the sunspot period that there can hardly be a doubt about the connection of cause and effect."

Jevons's ideas were spelt out in a second paper to the British Association, "The Periodicity of Commercial Crises and its Physical Explanation", and the first of two articles contributed to Nature. Although still concerned about the evidence on which his theory of crises was based, he had great confidence in its substantial truth.

Keynes, in his essay, listed the new discoveries which were his excuse for returning to his theory:

- (1) He had succeeded in carrying back the history of commercial crises at ten or eleven-year intervals almost to the beginning of the eighteenth century.
- (2) He was now advised by his astronomical friends that the solar period was not 11.1 years, as he had previously supposed, but 10.45 years, which fitted his series of commercial crises much better.
- (3) He now abandoned European harvests, the price statistics for which yielded negative results, as the intermediary through which sunspots affected business, in favour of Indian harvests, which, he argued, transmitted prosperity to Europe through the greater margin of purchasing power available to the Indian peasant for buying imported goods.

But apart from the coincidence of the periodicity of the commercial crises and the solar period, the causal link was never much stronger than a personal conviction that the decennial crises depended on meteorological variations of similar periodicity. His 1875 evidence, which depended on European harvests, was overturned in 1878 in favour of fluctuations in foreign trade resulting from cyclical crop changes in India and elsewhere. But even then the timing of the relationship was such as almost to suggest that the effect preceded its cause.

Later empirical work has largely discredited the theory that there is a link between business cycles and a 10 to 11year cycle in sunspot activity. Business cycles have recurred at shorter intervals than 10 or 11 years, and in spite of ingenious interpretations of harvest statistics over the years, it is now generally agreed that such an explanation could never completely account for the business cycle. Keynes's assessment is "Jevons's summed up as follows: notion, that meteorological phenomena play a part in harvest fluctuations and that harvest fluctuations play a part (though more important formerly than today) in the trade cycle, is not to be lightly dismissed."

## international news

As part of a desperate bid to get a grip on inflation, President Ford is proposing to prune nearly \$5,000 million from federal expenditures during the 1975 fiscal year, which has only seven more months to run. Welfare, health and veterans' benefit bear the brunt of the cuts, but biomedical research, space research and several nuclear power programmes are also in the running for some radical surgery.

Ford's proposals came in three chief parts. First, he asked Congress to take the knife to the Appropriations Bill for the Department of Health, Education and Welfare (HEW), which was then in the final stages of passage through the Congressional mill. Among the cuts Ford requested was a reduction of \$112 million in the budget for the National Institute of Health (NIH), a move which he said would reduce expenditures on new grants and contracts by 25% and cut continuation grants by 5%

Such surgery, he maintained, "could accomplished without seriously altering the momentum in biomedical research achieved by sustained and substantial growth in the National Institutes of Health in recent years", but since the NIH was hit hard by cutbacks in 1973 and received only a modest budget increase this year, few biomedical scientists will agree with that sanguine prediction. Congress, in

## **Cutting budgets** to fight inflation

by Colin Norman, Washington

any case, responded to the request by passing the HEW Appropriations Bill last week with a massive increase of \$256 million in the NIH's budget instead of a decrease of \$112 million, thereby putting the ball firmly back in Ford's court.

The Appropriations Bill would step up spending in every NIH institute, with the National Cancer Institute topping the list with a budget of \$692 million -an increase of \$164 million over last year. The National Heart and Lung Institute would also receive a massive infusion of funds, amounting to \$324 million, and the entire NIH budget would climb to \$2,090 million. The Appropriations Bill additionally provides a pot of money for biomedical training, which the Administration has been trying unsuccessfully to decrease.

Ford, it seems, has two options open if he is determined to press his case over the HEW budget. Either he can veto the Appropriations Bill to try to force Congress to reduce the amounts. or he could sign it and later propose that some of the increases be deferred until next year.

Finally, Ford is recommending that money which Congress has already allocated to some programmes be spent next year instead of this year. Among the programmes he has selected for such a stretch-out are several nuclear power projects, some space research and development, and solar and geothermal energy research supported by the National Science Foundation.

He is suggesting, for example, that \$80 million in the budget of the Atomic Energy Commission be deferred, including \$8 million earmarked for the breeder reactor, \$8 million for controlled thermonuclear fusion, \$4 million for environmental research and safety, \$6.7 million for development of hardware for the high temperature gas cooled reactor, and another \$13 million for equipment needed for the fusion programme. Although Ford maintains that the delays will have little lasting impact on the nuclear power programme, those proposals provide an important indication that the Administration may be losing some of its overriding enthusiasm for nuclear energy.

As for the space programme, Ford is requesting authority to withhold until next year some \$20 million earmarked for the joint USA-USSR docking mission. He also wants to defer expenditure of \$16 million for several space science and applications projects; and \$36 million from NASA.

THE six-year-old collaboration arrangement between the World Health Organisation (WHO) and the government of India, involving a research project on genetic control of mosquitoes, is due to expire in June next year. Following a controversy in India (Nature, September 20) over the American defence establishment's apparent interest and role in these projects, the the WHO agreement with the United meeting also recognised that considera-Indian government has been rethinking.

It is almost certain that the WHO will ask for an extension of its agreehave been completed by June 1975. (A big experiment aimed at controlling

Genetic Control of Mosquito Unit oured the creation of a separate 'monicharge of the Director-General of the from those not actually engaged in States government, to ensure that all tion of the possible future lines of the necessary safeguards are pro-development for the ICMR-WHO provided in its revised agreement with ject was the legitimate responsibility ment with India, because some of the the WHO-that is, in case it decides to of none other than the GCMU's own project's planned experiments will not extend the latter's mosquito research project committee. project beyond next June.

in all probability, insist that the tics, immunology and allergy, has fav-

(GCMU) be placed under the overall toring body' with a membership drawn Indian Council of Medical Research the project. This body, in close co-(ICMR) and that all project leaders operation with the concerned GCMU be appointed only with the specific staff, will monitor the effect and imapproval of the government of India. pact of all future releases of 'gene-The Indian government will also very tically manipulated' mosquitoes at all carefully examine various provisions of stages of the release operation. The

Meanwhile, at a meeting held last According to a note prepared by the month of the subcommittee set up by aedes aegypti genetically is scheduled Union Health Ministry on the GCM the ICMR's governing body to review to commence early next year.) Should project and placed before the Lok the administrative and technical conthere be an extension of the project, it Sabha (lower house of the Parliament), trol of the GCMU, it was felt that the will come only after the present agree- a recent joint meeting of the expert project leader should be asked by the ment with the WHO has been revised committee on virus and arthropod- Director-General (of the ICMR) to with new or amended provisions, borne diseases and geneticists from submit reports to him every 15 or 30 Among other things, India will, the expert committee on human gene- days about the work done in the unit.

from Narender K. Sehgal

sion on Environmental Pollution, a must for the next report. published last week (Cmnd 5780, • The cuts in British defence spending HMSO, £1.10).

Little progress seems to have been made on old problems like the control of pollution in estuaries, but the situation with others, such as the use of persistent insecticides in agriculture, seems to be improving and there are new problems, such as noise, which the

strict economic terms should be programme remains untouched. deferred. The commission comes out strongly against such arguments and feels that they should be "strongly resisted". "At the very least there should be permitted no further deterioration in environmental quality", it declares.

There is a useful section on 'who does what' setting out the structure of pollution control in the United Kingdom and the responsibilities of local authorities and the special statutory Wickramasinghe. bodies. The commission also notes the increasing influence that bodies such as summed up as one of youthful enthusi- intentions of the donors, some of the European Economic Community asm combined with a good deal of whom prescribed specifically that the are having and are likely to have on astronomical and mathematical ability, award was to be made regardless of British pollution legislation. A section Some of the more conventional studies nationality. Or sex.

better and worse, are reviewed in the Kingdom legislation and international stars and galaxies; at the other extreme Fourth Report of the Royal Commis- and European recommendations seems one member of the team is working

> will reduce military research and development expenditure by 10%. The detailed incidence of reductions is still

#### **Round Britain**

commission has picked out as a growing largely a matter of negotiation in diff, are supporting astronomy. In an threat. Twenty-nine million people in defence establishments and is not ex- interesting echo of remarks made reurban areas in Britain are expected to pected to be known for several months. cently by some other senior astronobe affected by 1980 through the growth Talk about increased coordination of mers in the United Kingdom, he of traffic noise above acceptable limits. research and development across mentions that there is great interest Inevitably there is concern that the NATO to save by avoiding duplication in astronomy at undergraduate level, environment could suffer unduly in the of expensive projects is largely mis- and says that there has already been present financial climate, and it will be guided; collaboration is already fairly an increase in enrolments in the argued that action on environmental extensive, so the cuts cannot be con-mathematics department, partly as a projects that cannot be evaluated in fined to rationalisation. The Polaris result of the astronomy courses now

CHANGES in the environment, for showing the interaction between United include aspects of the formation of on aspects of the Hoyle-Narlikar theory of gravitation, and another is investigating problems in quantum gravity and relativity, which include the possibility of adding time to the list of quantum variable parameters.

> It is hardly surprising that Wickramasinghe is full of praise for the way in which both the Science Research Council and University College, Caroffered.

• The creation of new, tenured posts in • This year's crop of medals from the astronomy at any university is an event Royal Society conforms to the pattern so rare these days as to merit attention of the past twenty years. Everything from the astronomical community goes to Britons and all except the two around the world. Just such a situation specifically for achievement in industry has occurred in Cardiff, where the De- (the Esso Medal is a newcomer this partment of Applied Mathematics and year) go to male Fellows. Sir Alan Astronomy at University College re- Hodgkin in his Presidential Address is cently moved to a new building and slightly defensive about the quality of expanded its astronomy interests, under the medallists in the light of "some the guidance of Professor N. C. mild sniping from Private Eye". The quality we don't doubt, but the narrow The atmosphere of the group is best national perspective goes against the

THE flight of Soyuz 16 (December 2-8, 1974) was specifically announced to have been a preparation for the joint Soyuz-Apollo programme scheduled for 1975. Observers of the Soviet space programme have interpreted recent Soyuz flights (including that of Soyuz 15 which somewhat coyly manoeuvred about the Salyut 3 space station, without actually effecting a link-up) as being a preparation for the joint programme.

The official purpose of the flight was described by the TASS agency as the "testing of on-board systems of the Soyuz craft, which have been modernised in accordance with the requirements of the joint flight, the carrying out of scientific and scientific-technical investigations, and also observation and photography of individual sections of the Earth's surface in order to obtain data for the solution of problems of the national economy." Leaving aside the final clause (which is included in all descriptions of Soviet

#### Preparing for that space link-up

from Vera Rich, London

space missions as a sop to the economic planners) perhaps the most interesting word in the whole release is "modernised"-which, to the student of Soviet semantics displays a refreshing honesty. At one time the word would surely have been 'adapted' and the implication would have been that in any international project it would not be the Soviet side which would have had to 'modernise' to meet the heeds of the other.

The experiments carried out by Soyuz 16 relate, in fact, both to the details of the link-up and to the requirements of the on-board systems. The life-support systems were tested and the medical checks made with a cabin pressure of 540 mm Hg, which suggests a working atmosphere rich in oxygen. Hitherto, the Soviet programme has preferred a natural atmospheric mix, so this implies something of a compromise with US standards.

Preparations for the link-up included an orbital transfer, on December 3, into a circular orbit at a height of 225 km and inclination 51.8°, described as "similar" to the one the Sovuz craft will have to adopt in the joint mission. In the course of the 32nd, 38th, and 48th orbits, the new link-up systems, "created in accordance with the requirements of the 1975 joint mission" were tested in toto and also unit by unit, the tests being monitored by cosmonauts Filipchenko and Rukavishnikov themselves, as well as by ground control. Simulated linkup tests were carried out satisfactorily.

The programme also included a number of astrophysical and biological experiments, the latter ranging from the exchange of micro-organisms to the effect of prolonged illumination on the cosmonauts.

## correspondence

#### Entry forbidden

SIR,—This year I had planned to attend the Ninth International Meeting of FEBS (the Federation of European Biochemical Societies) in Hungary. I am a scientist, I paid my registration fees and I even had flights and accommodation arranged; but I was barred on grounds of nationality. I am Israeli!

I suppose the word international has a different meaning in Hungary.

A PhD student at King's College, London, and a member of the British Biochemical Society, I acted in accordance with the instructions in the booklet issued by the Hungarian organisers, "... Participants from countries that do not have diplomatic relations with Hungary should apply for a visa to a General Consulate of Hungary in a European country they find most convenient", and applied for a visa from London.

Even though they had four weeks to issue the visa and in spite of a special request from Professor H. R. V. Arnstein, Secretary of FEBS, they kept me waiting until the very last moment. It was intimated that I could pick up the visa when I stopped in Vienna on my way to the conference. But when I arrived at the Embassy in Vienna, three days before the conference, I received a rude and cold reception. After keeping me waiting for half a morning, they told me I was on a "black list" and forbidden entry to Hungary.

This left me in Vienna, holding worthless train and hotel reservations, wondering about the purpose of international conferences.

I would say to all organisers that if their conferences are to be truly international, they should choose countries which foster this spirit, not, for example, Hungary.

Yours faithfully,

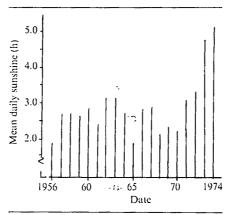
S. PELLER

King's College, London WC2, UK

#### Cameroon climate

SIR,—The catastrophic effects on humanity of the major climatological fluctuation which has caused so much concern in the Sahel zone of Africa may have overshadowed other smaller changes in the meteorological records recently obtained in adjacent areas.

Sunshine records obtained during the past 19 years in Yaounde and published by the National Meteorological Bureau of the Cameroon indicate, perhaps, a considerable shift in the distribution, and thus the total amount, of daily insolation during some agriculturally important months of the year. For example, the mean daily number of sunshine hours for July in the years 1959–74 is illustrated in the accompanying graph. The difference in the years



1973 and 1974 by comparison with the previous distribution of approximately 2 to 3 hours a day could, if confirmed by subsequent records, possibly affect the agricultural productivity of the region, which relies heavily on cocoa, a crop noted for the complex relationships between growth/yield and the shade factor.

The way in which the production of dry matter depends on energy provided by insolation is well known and if the radiation available for agricultural production has altered over a wide area, the aid programmes planned by donor agencies may need revision.

J. DANCER

Ecole Nationale Supérieure Agronomique, BP 138, Yaounde, Cameroon

#### Asbestos and health

SIR,—In Peter J. Smith's article (October 18) the point is well made that scientists should be more concerned with the practicalities of occupational health. We think it a pity, however, that he should have selected asbestos as an example of a health problem which scientists have ignored, since more scientific effort has in fact been expended on this problem than on most other similar problems in recent years, as the literature testifies.

It is not true that "asbestosis is taking an increasing toll" in this country. The number of new cases diagnosed may be on a higher plateau now than it was in the 1950s, reflecting factory conditions long ago and the increased usage of asbestos in an uncontrolled way in, for example, insulation, but since reaching a peak of 168 in 1967 there has been no further increase. The average since then has been 139 per year. We have every confidence that stringent controls associated with the Asbestos Regulations 1969, which apply to all asbestos work, will in coming years reduce these numbers to a very low level indeed. Indeed they are intended to eliminate them altogether.

The standards associated with the controls have a scientific base for which scientists working for government departments, the Medical Research Council, the British Occupational Hygiene Society and the industry-sponsored Asbestosis Research Council deserve full credit.

Yours faithfully,

W. P. HOWARD

The Asbestos Information Committee, London W1, UK

#### Academic consultancy

SIR,—Your editorial "Academics in the Boardroom" (November 22) implies that consultancy by university staff is something to be encouraged, whereas in fact it is something to be deplored.

Professors and lecturers at universities are in receipt of a full salary for a full-time job of teaching and research, and none of them has any right to use any part of that time in consultancy to anyone, whether industrial firm or individual, or even government department or agency, for the benefit of his client or himself.

In addition no member of university staff has the right to use apparatus and facilities provided at public expense for the purposes of teaching and research, for the benefit of any organisation or for his own benefit.

It is time that the Department of Education and Science clamped down on such use of public funds to subsidise such consultancy, which has forced so many independent consultants out of business. How can any independent consultant possibly compete with the university member who does not have to pay for his equipment and facilities or premises, and may even use students as unpaid staff?

Yours faithfully, H. A. Cook

14 St Alban's St, London SW1, UK

## news and views

### Excuse me, your slip isn't showing

THE inexorable motion of plates relative to each other at the Earth's surface leaves behind a magnetic record at ocean ridges where new plate is formed. Much of the early work on plate tectonics was devoted to using this record together with a knowledge of geomagnetic field reversals to measure relative plate motions. By 1970 most geophysicists were agreed on the values for inter-plate velocities, typically a few centimetres per year. The motion was obviously manifested, in fits and starts, by earthquakes. But a most important question remained—did the rate determined as an average over several million years tally with the present rate determined from the cumulative displacement of successive earthquakes? For if not, either the movement had a cyclic character, or some of the strain build-up was relieved by non-violent processes such as creep on the fault plane. The relevance of all this to earthquake prediction efforts is obvious.

In 1971, Davies and Brune (Nature phys. Sci., 229, 101-7) made a major contribution to resolving this question. The only parameter of earthquakes which has been reliably measured over the last seventy years is magnitude. The logarithm of the amplitude of the radiated surface waves (corrected for distance), or surface wave magnitude,  $M_s$ , is a rough and ready measure of the slip on the fault plane once some assumptions have been made about the dimensions of the fault surface. These assumptions, it is widely admitted, are fairly major. Davies and Brune considered the magnitudes of very large earthquakes on all plate bound-

aries and concluded that the inferred displacements over the last seventy years agreed rather well with plate tectonics predictions. Thus it was possible to deduce from their results that for most plate boundaries the part that was played by, for example, creep was certainly not a dominant one. The Mediterranean region did not immediately fit into this scheme. For one thing the tectonics of the area is immensely complex so it is not easy to talk about a small number of plate boundaries. For another, the area is not highly seismic so the data are fewer (the frequent damaging earthquakes in the region result more from the proximity of people to faults than from very high magnitude events). The area clearly warranted a special study and this has now been done by North who reports in this week's *Nature* (page 560).

He comes up with a most interesting conclusion. When similar techniques to those of Davies and Brune are applied in the Mediterranean, the movement on most plate boundaries inferred from earthquake magnitude is very much less than plate tectonics would have it. Either strain is being built up throughout most of the region (and nowhere else in the world) on various types of fault—or strain is being relieved in the Mediterranean area by other processes than major earthquakes—and again this is not happening elsewhere in the world. It is a provocative and important result and should stimulate more research both in the field and in the laboratory.

DAVID DAVIES

## Switching off symmetry breaking

The elementary particles nearly obey a number of simple and elegant symmetry rules. Many useful approximate calculations can be made by assuming that the symmetries are perfect-for instance by assuming that the decays of K° mesons are well behaved when all the particles in the decay process are regarded as travelling backwards in time (time-reversal symmetry), or by assuming that the proton and neutron have the same interactions as the lambda, sigma and xi hyperons. Recently a great deal of progress has been made in building field theories of the elementary particles, such as the unified weak and electromagnetic theories which have been supported by the discovery of 'neutral weak currents' (see Nature, 245, 119; 1973 and 250, 186; 1974). These new theories incorporate symmetry breaking as a 'spontaneous' effect, drawing on successful theories of low-energy solid-state phenomena such as superconductivity which is also caused by 'spontaneous' symmetry breaking.

When a superconductor with zero resistance is placed in a high magnetic field, above some critical strength, it reverts to being a normal conductor. The theory of this transition has been taken as a model by Salam and Strathdee (this issue of *Nature*, page 569). They show that the same kind of behaviour could occur for a number of the symmetry-breaking effects in the weak interactions of elementary particles. For instance, there may be a critical magnetic field which would turn off the time-reversal asymmetry in  $K^0$  decay. They estimate this field to be between  $10^8$  and  $10^{14}$  gauss— $10^4$  or more times what can be achieved in a practical apparatus. Another example they give is that the  $\beta$  decay of the lambda hyperon may be turned off at  $10^{18}$  gauss.

Although such field strengths may be difficult to achieve in the laboratory—partly because of the critical field effect itself in superconductivity which limits the strength of our magnets—they may already exist inside pulsars. If so, it may be possible to devise ways of detecting the effects of an absence of symmetry breaking. Another way of achieving high field densities may be through laser compression, using similar techniques to those being developed in research on thermonuclear fusion.

Even if we cannot achieve the predicted fields, they urge that experiments should be started to look for this kind of effect. Their current calculations are so model-dependent that they cannot rule out new effects at fields as low as 10<sup>6</sup> gauss.

D. J. MILLER

## Chromosomes and malignancy

ABNORMAL chromosomes are a regular feature of tumour cells. Non-transformed cells rarely show chromosomal abnormality unless obtained from developmentally defective individuals, many of whom show an increased predisposition to cancer. Altered chromosomes therefore either predispose oncogenic change or accompany it. Most cancer-producing agents cause chromosome damage, which may therefore be an important aspect of their potency for tumour induction. But the majority of such damaged cells are inviable, a few are transformed and only a proportion of these may have malignant properties. These wide ranging manifestations of the transformed state have attracted the attention of many workers seeking some explanation in terms of the accompanying chromosomal alterations.

Sachs and his group (Hitotsumachi, Rabinowitz and Sachs, Nature, 231, 511; 1971) have shown that the in vitro propagation of polyoma transformed cells may be accompanied by loss of malignant characteristics and the appearance of new groups of chromosomes which are suggested to carry suppressors of malignancy; the balance of these chromosomes in the nucleus is supposed to be an important feature of its control. Harris and his coworkers (Klein, Bregula, Weiner and Harris, J. Cell Sci., 8, 659; 1971; Weiner, Klein and Harris, J. Cell Sci., 12, 253; 1973) have fused together cells with and without malignant properties. The result of such an experiment is usually suppression of malignancy, followed by its restoration after the loss of chromosomes from the normal, non-malignant, parent. Fusing together two malignant cells however usually resulted in a malignant cell without the necessity for chromosomal loss. In certain cases the latter experiment resulted in loss of malignancy (Harris, Proc. R. Soc., B179, 1; 1971), all of which leads Harris to stress the role of individual chromosomes which carry malignancy suppressors as the important feature of its control. Both groups invoke chromosomally based factors as the essential elements, although in neither case are particular chromosomes designated as the carriers.

In this issue of *Nature* however (page 610) Codish and Paul report the reversible appearance of a specific chromosome which suppresses malignancy in a chemically induced tumour. When present in two copies, both malignancy and antigenicity are suppressed, but both are restored when only a single copy is present. The extra chromosome is thought to originate from the fusion of part of a normal number 19 mouse chromosome with an unaltered number 7. Since the normal elements of this marker chromosome are also present in the cells, it seems possible that the suppression involves a position effect following fusion of the two normal chromosome segments, although this is not suggested by the authors. There seemed to be no other consistent correlation with suppression of malignancy.

A somewhat similar mechanism, resulting in the acquisition of malignancy rather than its suppression, is suggested in the case of the now classical examples of human tumours: chronic myeloid leukaemia in which 90% of cells exhibit a loss of most of the long arm from chromosome 22 (Caspersson, Gahrton and Lindsten, Exp. Cell. Res., 63, 238; 1970) and the acquisition of a similar fragment by the number 9 chromosome (Rowley, Nature, 243, 290; 1973); and meningiomas in which there is also loss of part of the number 22 chromosome (Zankl and Zang, Humangenetik, 14, 167; 1972). Other cases of specific abnormalities include the number 14 chromosome with an additional terminal fluorescent band in tumours of some patients with Burkitt's lymphoma (Manolov and Manolova, Nature, 237, 33; 1972), and the number 13 interstitial deletion in retinoblastoma (Wilson, Towner and Fujimoto, Am. J. hum. Genet., 2, 212; 1973). Not surprisingly, some tumours seem not to exhibit specific alterations in chromosomes, but show random changes (Popescu et al. Int. J. Cancer, 14, 461; 1974) and others exhibit variability in malignancy correlated with the fluctuation in numbers of heterochromatic minute chromosomes (Shepard et al., Cytogenet. Cell Genet., 13, 279; 1974). The interest of the material described by Codish and Paul lies in the apparently clear-cut nature of the phenomenon and one hopes that they will take advantage of the cell fusion approach to analyse it in more detail.

The tendency of certain tumours to exhibit predictable alterations in certain chromosomes, whether connected with increased malignancy or its suppression, presumably reflects a number of related factors such as the probability of the alteration occurring, the viability of the altered state and the predilection of certain carcinogens for causing damage at selected sites. It may be that certain chromosomes (for example those involved in nucleolus organisation) come more frequently into contact than others thus increasing the probability of interchange.

It seems quite certain however that particular groups of chromosomes are more frequently involved in formation of the marker chromosomes typical of a cell line than are others following virus transformation by adenovirus, SV40 and herpesvirus (Gallimore, cited in McDougall, Prog. med. Virol., in the press). Chemical transformation of rat cells also results in the preferential involvement of three autosomes in marker formation (Olini and DiPaolo, J. natn. Cancer Inst., 52, 627; 1974) and two of these are regularly involved in the genesis of markers in adenovirus transformed cells (McDougall, Prog. Med. Virol., in the press). An important future task will be to establish the mechanism of this effect. For this purpose virus-transformed cells seem ideal since they permit the use of specific probes which can detect integrated viral genomes, their transcriptional and translational products at the cell level. A beginning to such work has been made by the localisation of the control of SV40 T antigen to human chromosome 7 (Croce, Girardi and Koprowski, Proc. natn. Acad. Sci, U.S.A., 70, 3617; 1973). Progress should be spectacular in view of the recent advances in analysis of the transforming genes of adenoviruses (see Rogers, Nature, 251, 668; 1974). K. W. Jones

## Arrival of the age of Rama

It is now generally accepted that the survival of the human race is threatened by twentieth century technology. Mankind must live with the uncertain hope that future imnovation will not create more dangers than it removes, and occurs rapidly enough to forestall the collapse of organised industrial society.

Speculative science fiction enthusiasts have long dreamed of preserving humanity from earthbound destruction by colonising other planets. The stunning successes of near space exploration over the last decade have moved the discussion of extraterrestrial migration out of the domain of pure fantasy into the 'light relief' area of established science. But most of the colourful accounts published assume a depressingly high level of technology, in many cases decades or even centuries ahead of our present development. It is not now at all clear that technology will ever be able to reach the level at which exotic extraterrestrial solutions, such as the colonisation of Mars, will provide for the continuation of the human species. The global crisis has apparently arrived a few decades too soon.

If there is little ground for confidence in further gigantic technological leaps in the foreseeable future, it is heartening

to learn what radical possibilities for the extraterrestrial propagation of the human race are available with present day technological, economic, social and cultural constraints. One such possibility was put forward in Nature (250, 636; 1974) and received a detailed appraisal in Physics Today (27, 32; September 1974). In an article entitled "The Colonisation of Space", Gerard K. O'Neill, a professor of physics at Princeton University, outlines a scheme for constructing small "planetoids" in space for habitation by millions of people. The structure of these artificial worlds will be familiar to all those who have read Arthur C. Clarke's novel Rendezvous with Rama. They consist of a rotating cylinder, with the inhabitants living on the inside surface at normal gravity, in a completely self-contained ecosystem. The cylinders would be lit by sunlight reflecting from mirrors through glass or plastic cellular windows. Otherwise construction would be mainly from aluminium and titanium, with power requirements more than adequately met from solar radiation using conventional steam turbine electric generators.

Cylinders could be constructed in tandem, oppositely rotating and coupled so that they may always be orientated towards the Sun. A suitable place in space to locate them would be the L<sub>4</sub> and L<sub>5</sub> Lagrange libration points of the Earth-Sun-Moon system.

At first sight, Professor O'Neill's proposals appear to suffer from all the drawbacks of traditional space colony speculation; principally, the enormous energy expenditure necessary to transport a vast mass of material by rocket from the surface of the Earth. In fact, two crucial aspects of his ideas remove this objection. First, the project is selfperpetuating. With the establishment of a small scale pilot scheme providing an adequate environment for an industrial base, components for much larger cylinders could be manufactured and assembled in situ, thus reducing the problem to that of providing only raw materials. Second, the source of raw materials is suggested to be the Moon (and eventually the asteroid belt), the low gravity and vacuum conditions of which reduce enormously the energy expenditure required to transport ore to the colony. O'Neill discusses novel mechanisms, such as a two-arm rotor, the ends of which are moving at lunar escape velocity, which would propel. pellets of lunar material at the rate of 3.250 tons per year, whilst operating at only 1,600 horse power. After a generation or two, the cylinder worlds would become entirely self-supporting-

In spite of the enormous possibilities available for this type of adventure using only current technology it is hard

to resist the impression that space Goliaths of this kind would not be economically viable. O'Neill has undertaken a cost analysis of the scheme. and claims that a kilometre-long, 10,000 population cylinder would cost about the same as the Apollo space project, thus being well within even national resources, and could be established as early as 1988. The cost of transporting 10,000 people to the colony could be met by the transportees themselves at, say, £10,000 per head. Larger cylinders would be able to pay for their own construction out of various space-based industrial products, such as high strength crystals.

Looking beyond the Solar System, there are available possible mechanisms for propagating humanity across the Galaxy. Although current rocket power can only achieve payload velocities of a few thousand miles per hour, travel time to nearby stars in the Galaxy is still only a few million years, that is, comparable with the age of the human race and a minute fraction of the life-time of most stars. Naturally, adult humans could not accompany a space capsule on such a venture, but millions of fertilised ova could easily be sent in a very small craft: The chief problems would be biological rather than mechanical. Craft such as these could then search the Galaxy for likely looking planets. One scheme might be to attempt to attract hypothetical extraterrestrial civilisations, which would then be given the responsibility of 'hatching' the nascent community. Alternatively this could be done automatically. The craft could be equipped with detailed biological and cultural information, so that the scheme would amount to a true extension of the human race rather than just putting flesh and blood on other worlds.

In the present social and economic climate, it is hard to imagine that schemes of this variety would get off the ground. The failure of space technology to provide any convincing major benefit to mankind has led to a rejection of large scale space exploration by the taxpayer. While space programmes were orientated towards 'glory trips' this was bound to happen. But against this background of muddled priorities projects such as O'Neill's are very appealing. Many direct benefits to mankind are apparent, not the least of which is a solution to overpopulation. Such projects could be undertaken as essentially a human, rather than a national venture, and provide a new perspective on the position of man in the Universe. They would also give a strong stimulus of confidence in a maligned technological system which, whether we like it or not, we can no longer do without. P. C. W. DAVIES

## A centenary in population biology...

from J. L. Harper

In 1874 Wilhelm Carl Naegeli presented a paper to the Munich Academy of Science on "the displacement of plant forms by their competitors" (Sitzb. Akad. Wiss. Munchen, 11, 109; 1874). This represented perhaps the first attempt at a serious mathematical treatment of one of the fundamental questions in population biology and arose directly from Naegeli's reading of Darwin's Origin of Species. Publication of Naegeli's paper in the mathematics/physics papers of the academy was probably partly responsible for its failure to have any impact on the development of biological thinking. The paper is summarily mentioned by Gause (The Struggle for Existence; Williams and Wilkins, Baltimore, 1934) but otherwise seems to have been ignored in the development of a science that gained no significant momentum until 50 years later. Remarkably, this early essay in modelling of populations concentrated on problems in plant ecology whereas virtually all the subsequent development of experiment and theory in population biology has been concerned with animals.

Naegeli considered the consequences of introducing a new form into an already fully stocked population of plants. This contrasts with later developed theory which was usually concerned with simultaneous colonisation of an unoccupied habitat. He suggested that in a locality the average number of plants (z) of any type reveals its relative strength against all other competitors. The average age of the individual plants (d) and the annual replacement by young plants (e) together determine (z), both (d) and (e) depending on heredity and environmental influences. He recognised that when two species differ markedly in size, their numbers may be an inadequate description of relative abundance and the relative space occupied by the species might then be a better measure, but he avoided this problem by comparing pairs of closely related species. His ideal model was of a community of only two such species but "this very simple case . . . seldom occurs in nature". The seemingly more complicated but analogous case in which two closely related taxa live among vegetation composed entirely of other kinds of plants could be studied if the species (a) and (b) make the same or almost the same demands on other species in the community and compete in the same way as each other. In such a model (a) and (b) compete against each other but in a mutually exclusive way-sharing a total number of

individuals (a+b) in proportion to their competitive vigour. (This treatment comes close to a formal definition of the 'niche'.) The question that Naegeli posed was: what changes occur in any given number of individuals, how do they succeed one another and what state of permanence do they achieve when certain assumptions are made about the length of life and conditions of replacement in both forms?

His model develops from z/d+ $z_1/d_1=e+e_1$  where z/d and  $z_1/d_1$  are the annual wastages of the two forms (a) and (b) respectively and e and  $e_1$  the corresponding new colonists: e and  $e_1$  are variable but constant in their relation to one another. Naegeli used iterative procedures to show the simple consequences of varying parameters but historically the most fascinating part of the paper is his choice of special cases that he considered biologically relevant.

In the simplest model he assumed (1) that the life span of both forms and the ratio of their replacements solely on supposedly constant hereditary and ecological factors and that the values of  $d+d_1$  and  $e+e_1$  are independent of one another of  $z+z_1$ . He noted however that (2) the life span of a form may depend on its numbers or the numbers of its competitor (that is, density-dependent control of life span). He modelled the life span of a dependent on the numbers of a; on the numbers of b; and on the numbers of a+b.

He also modelled the three comparable situations (3) in which the annual replacement rate is dependent on the number of individuals. He envisaged positive effects such as the presence of older plants protecting seedlings and negative effects when the presence of many old individuals deprive young

Toehole for a toad

growth of some limiting nutrient. Alternatively (4) the annual replacement of one form may be altered by the numbers of other forms or (5) the annual replacement of either form can be altered by the numbers of both forms acting together. He also considered interactions between the replacement rate and the life span: (6) the annual replacement can be modified by the life span of the individuals of the same form or (7) of the other form.

The essay uses the twentieth century concepts of density dependence and frequency dependence (though not the words). The models are deterministic but he was aware of the significance of chance events and noted the high chance of random extinction of a species when its frequency balances at very low numbers. Many of the interactions he chose to model were nonlinear and he was particularly interested in models in which the square root of density was considered. He concluded that there were very many model situations in which even closely related species would not exclude one another. Today after an era in which most population biologists have struggled to explain diversity, it is curious to see the argument inverted with mutual exclusion regarded as the case needing explanation.

It was a remarkable vision which saw that the outcome of a struggle for existence depended on the comparative effects of intra- and inter-specific competition, that the effects might not be linearly related to density, that (though he did not develop the point further) it was practicable to define the effect of the density of one species by some factor into the effects of the density of another, and that the probability of extinction was relevant when numbers became small.

Naegeli had very great influence on botanical thinking in the nineteenth century. He made important studies of algal structure and life cycles and published classic early work on plant anatomy and ultrastructure. He contributed much to the debate on evolutionary theory and studied the mechanism of inheritance (unfortunately choosing the apomictic Hieracum as his experimental material). All of these activities are reported in the long obituaries by Vines (Proc. R. Soc., 51, 27; 1892) and Scott (Nature, 44, 580; 1819) and in the outline of his life in Gillespie's Dictionary of Scientific Biography (9, Scribner, New York, 1974). None of these even mentions "The Displacement of Plant Forms by their Competitors". The paper must, in 1874, have seemed quite irrelevant to what biology was all about. In 1974 it has little to contribute to advancing ecological science but holds a fascinating place in the history of ideas.

## ... and a bicentenary for science writing

from Peter J. Smith

OLIVER GOLDSMITH, who died just 200 years ago, is one of those unfortunate writers whom most modern critics find it fashionable either to denigrate or ignore, but whose literary stature remains obstinately undiminished among a wider public. Admittedly, his masterpieces were few; Goldsmith's fame rests largely on one poem, The Deserted Village, one novel, The Vicar of Wakefield, and one play, She Stoops to Conquer. He was above all and by necessity a hack writer, though one who peformed with skill.

By far the most important piece of hack work Goldsmith turned out was his An History of the Earth, and Animated Nature (1774), a remarkable eight-volume study. If it seems strange to find a poet, novelist and dramatist writing with some authority on a scientific subject, it needs only to be remembered that in the eighteenth century the disastrous split between science and the humanities had not developed to its present state. Moreover Goldsmith had studied medicine in Edinburgh and Leyden and would thus have had some feeling for the scientific methods of the day. As a writer to order he would in any case presumably have been willing to turn his hand to most subjects. Samuel Johnson is recorded as saying that if Goldsmith could distinguish between a cow and a horse that was about as far as his knowledge of animate nature went, although this flippancy was later to be balanced by the more serious admission that Goldsmith "has the art of compiling, and of saying everything he has to say in a pleasing manner. He is now writing a Natural History and will make it as entertaining as a Persian

So what was the nature of this compilation? The last seven of the eight volumes, which are concerned with the "animated nature" of the title, I leave to the naturalists to judge. Of particular interest to Earth scientists, however, is the first volume which is complete in itself. Goldsmith's purpose in this volume was to give a comprehensive view of the Earth and its physical, inanimate characteristics, which he does in the form of a progression from the globe as a whole ("a sketch of the universe") to more localised features such as mountains, rivers, tides and winds. On the way he deals with the Earth's surface, its internal structure and theories of its formation; he describes fossils, earthquakes and volcanoes; he speculates on the formation of new islands, the origin of rivers and erosion by the sea; he discourses

on air, meteors and the saltiness of the oceans. In short, he gives a general and accurate picture of how the Earth was viewed in the late eighteenth century; and he does so, moreover, within a logical framework which most writers of books about the Earth have adopted ever since.

As Goldsmith freely admits, the model for his first book was Buffon's Histoire Naturelle; but whereas Buffon was out to establish his theory of the Earth above all others and was quite happy to make some of the facts fit his case. Goldsmith had no axe to grind. He was concerned to be more critically objective and discriminating, and thus went beyond Buffon to Burnet, Woodward, Whiston and other sources, both acknowledged and unacknowledged. But from wherever his information came, his judgement was always on the alert and he was ever ready to discard the opinions of others, however eminent, if they were refuted by personal observation.

Goldsmith never lost sight of his intention to write not a scientific treatise but a popular account; his concern was to produce "innocent amusement for those who have nothing else to employ them, or who require a relaxation from labour". The result is a book which is both scientifically accurate (for the time), and a work of genuine literary merit written with what Jeffares (Oliver Goldsmith, British Council, 1959) calls "all the ease and grace we expect from Goldsmith". In seeking "to drag up the obscure and gloomy learning . . . to open inspection" rather than to publicise scientific advances or make a scientific case Goldsmith must be regarded as one of the earliest of what are now called 'science writers'.

During the following century, Goldsmith's An History of the Earth, and Animated Nature went into at least 23 editions, including one in Welsh. His model was copied by scores of writers during the nineteenth century. Could it be that Goldsmith's science writing and its legacy, ignored by scientific and literary historians alike, actually affected the public mind more than many of the better known purely literary works?

## A night on Mount Hamilton

by John Gribbin

THE Lick Observatory on Mount Hamilton, near San Jose, has an interesting history and a fine array of telescopes which make it well worth a visit from any itinerant astronomer (or ex-astronomer) who happens to be in the neighbourhood. I was in that happy position on October 25, and my native

guide, Dr John Faulkner, was also able to provide a potted history of the observatory. (For further details see the booklet *Lick Observatory*, fifteenth edition, available from the University of California, Santa Cruz.)

It seems that James Lick, who provided the finance for the building of the observatory, now rests in a tomb underneath the 36-inch refractor. He originally intended that the memorial by which the good citizens of San Francisco should remember him would be a pyramid downtown, rather larger than that of Cheops. He was dissuaded from this by a retired sea captain who pointed out the great lustre that could be brought to the name of Lick by an observatory containing the largest telescope in the world. After a costly site survey covering much of the United States (and the first of its kind) Lick and his seafaring friend were no doubt delighted to find that Mount Hamilton, in their own backyard, was as good a site as any available.

Building an observatory on the top of a mountain was an epic achievement in the 1870s. The builders did, in fact, cheat a little on Lick's bequest, since they did not feel up to the task of building a reflector bigger than the Earl of Rosse's remarkable Irish instrument. Instead they built the largest refractor in the world.

Since those days, the Lick Observatory has expanded, and so, alas, has the nearby town of San Jose. The pretty lights below the mountain and the haze from the urban environment conspire to plague the astronomers. But although there is talk of a new observatory being built in a less accessible part of California, there are no definite plans as yet.

Pride of place on the mountain today is taken by the 120-inch telescope, which the Lick observers claim to be the best light bucket in the world. The impressive array of electronic gadgets which can be attached to it makes the telescope, they claim, a better performer even than the 200-inch at Palomar, although the Lick instrument has just 36% of the collecting area of the larger telescope. But they may soon have to consider a new rival to the telescope which can get the most in-

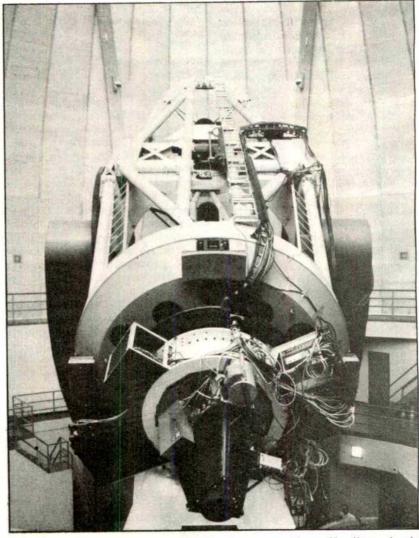


Fig. 1 The business end of the 120-inch telescope on Mount Hamilton, showing the impressive array of electronic instrumentation. (Courtesy Lick Observatory.)



Fig. 2 "It was a dark and stormy night . . ." View of the main building of the Lick Observatory from close to the 120-inch site. The small hut in the middle ground conceals the absence of any electronic instrumentation on the Tauchmann.

formation out of the smallest number of photons—the Anglo-Australian Telescope, whose director, Jo Wampler, must have taken with him from California many of the tricks of the trade.

But the casual passer-by is not allowed even to enter the 120-inch dome, much less operate the mighty machine. For such itinerants, a slightly less sophisticated instrument with an aperture one sixth that of the big telescope is available. This instrument, the Tauchmann reflector, provided the entertainment on the night of my visit. In the words of one recent user it provides "a rickety but fun operation". The meaning of this can best be gauged from the instruction book provided to aid the unfamiliar with their observations. After explaining how to open the dome (not as easy as the uninitiated might guess) and how to set the telescope in the required direction, some handy hints are provided to explain why you may not be able to see anything. The list begins: "(1) Is telescope pointing out of dome slit?" and continues in similar vein.

Our fun was only slightly marred by the 99% cloud cover on the night of October 25. We did, briefly, see the Moon and my colleague claims that he saw Jupiter, but the observation was not confirmed. As the only observers on the mountain that night we can at least report that for once the Tauchmann achieved more than the 120-inch. Of course after a two hour drive back to Santa Cruz on the coast we found a cloudless sky, thus proving Parkinson's second theorem.

#### **Chunky sputtering**

from Robert W. Cahn

THOSE incorrigible optimists who look for salvation to the large-scale controlled release of thermonuclear fusion energy should be cheered by a conference which was held at Argonne National Laboratory near Chicago last January, under the title Surface Effects in Controlled Thermonuclear Fusion Devices and Reactors. The proceedings have now been published as a special volume of the Journal of Nuclear Materials. For perhaps the first time, a major conference took as a premise that large fusion reactors will be built well before the century is out; Klaus Zwilsky of the Division of Controlled Thermonuclear Research of the United States Atomic Energy Commission proffered a timetable which included an experimental power reactor in operation by 1984. The conference was concerned with one class of engineering problems which will arise in the design of such reactors, namely the interaction between imperfectly confined plasmas and the wall of the containing vessel, combined with more conventional radiation damage due to energetic particles, especially neutrons, impinging on the walls. Physicists and metallurgists do not lavish time and money on such difficult studies unless hard-headed engineers have concluded that the hardware is in principle feasible.

Many of the papers dealt with the effects of particles peculiar to thermonuclear reactors, such as lithium, deuterium and tritium ions, but a particularly intriguing group of papers was concerned with that familiar entity, the high-energy neutron. The neutrons impinging on a fusion reactor wall will be exceptionally energetic, and little is known concerning the effects of such super-neutrons. Kaminsky and Das (J. nucl. Mater., 53, 162; 1974) experimented with 14 MeV neutrons from an accelerator, impinging on niobium. It has long been realised that the sputtering (that is, collision-induced erosion) of the metal could gradually damage the wall, and perhaps more serious, contaminate the plasma with metal ions through the knocking-out of individual metal atoms. But something else quite unexpected happened as well. 'Chunks' of niobium often exceeding one micrometre in size and containing many millions of atoms, were emitted from the surface. Nothing remotely like this had been reported before in sputtering experiments. Kaminsky and Das discovered that the emission of chunks was much greater when the target was cold-rolled and roughly polished than when it was annealed and finely polished; though

these aspects were not varied independently, the investigators were sure that internal stresses and rough surfaces each and severally promote emission of chunks (as opposed to single atoms).

A companion paper by Guinan (ibid, page 171) develops the hypothesis that thermal transients engendered by collision cascades within the metal will generate shock waves which might suffice to spall off chunks. Guinan concludes that the magnitude and duration of such shock waves would not alone suffice to do the trick. But if the metal contains regions of high internal stress (for instance, at the head of a dislocation pile-up) then as Guinan shows, a shock wave can act as a trigger to release the stored internal energy, leading to the ejection of a chunk. On this hypothesis, as Karminsky and Das have found, both plastic deformation and surface roughness will foster chunk emission (though it must be admitted that the role of the second variable is not clearly explained). Unannealed cold-rolled foils, which contain macro as well as micro stresses, are particularly vulnerable. Karminsky and Das conclude that a high mirror polish on a vacuumannealed metal, monocrystallic for choice, will render it virtually immune to chunk emission or the associated damage. It seems the phenomena resembles ordinary fatigue, very largely controlled by surface quality.

In related studies, Blewitt et al. (ibid., page 189) failed to find chunks emitted from gold bombarded with 14 MeV neutrons, whereas Biersack, Fink and Mertens (ibid., page 194) found copious chunk emission when UO2 films were sputtered by selfgenerated fission fragments during neutron irradiation. Here there was a pronounced effect of foil thickness (thin foils emit more chunks), and in particular, there was a lower limit of neutron dose below which no chunks emerged. It is surprising that the many earlier experiments on fissionfragment self-sputtering had not previously uncovered this phenomenon.

The notion, which seems well supported by the experiments on niobium, of the cold-rolled metal as a sort of self-stressed microbomb from which fragments are released by neutron bombardment, is reminiscent of researches at Harwell in 1956 by Cottrell and Roberts, who discovered that the intergranular stresses engendered by uranium by the anisotrop radiation-induced growth of individual crystal grains led to creep / absurdly small applied stresses the external stress triggers/ release of internal stresses prolonged uranium fissio. creep has come to be rec

major hazard in fission reactions, and it may be that chunk emission must be guarded against with equal care in the fusion reactors of the future.

#### Cancer in Tuscany

from R. A. Knight and N. A. Mitchison Banners decked the streets of Florence, Sienna, Montecatini, Pisa and Lucca, for the 6,000 members who came to listen to papers given at the Eleventh International Cancer Congress by some 4,500 authors on October 20-26. Buses and trains shuffled to and fro between the five cities, but we found the delight of autumn in Tuscany compensated for the fuss. The congress accurately conveyed the flavour of cancer research over the past four years: greatly to oversimplify it was one of slow progress in cell biology and immunology, with dramatic news concerning viruses. We listened to the virology and immunology papers.

In the RNA tumour virus field, new work localised specific viral antigens on the tumour cell surface. D. P. Bolognesi (Duke University) showed that monospecific anti-p30 and antigp71 sera were cytotoxic for virus producer and non-producer methylcholarthene induced mouse tumours, although the anti-p30 effect may be mediated by antigen non-specifically absorbed to the cell surface. Natural mouse antibody is also cytotoxic for these cells, and can be partially absorbed both by gp71 and by p30. The predominant anti-viral antibodies in the serum of normal wild and laboratory strain mice, reported by R. C. Nowinski (University of Wisconsin), react with p15, and with both viral envelope glycoproteins. Nowinski also finally laid to rest the old dogma that mice cannot mount a humoral anti-p30 response, since this is the major specificity in the serum of C57BL mice immunised with the K36 leukaemia.

Several workers discussed the relationship of oncogenic viruses to human tumours. G. Klein (Karolinska Institute) reported that although 98% of African Burkitt lymphoma biopsies were positive both by nucleic acid hybridisation for EBV DNA, and for the Epstein-Barr nuclear antigen, European cases were negative for the genome. S. Speigelman (Columbia University) summarised evidence from his laboratory for an association between 70S RNA and a reverse transcriptase-containing. particle in a variety of human tumours, and showed that this agent contained a protein which cross reacts with p25 from the Mason-Pfizer agent. R. C. Gallo (National Institutes of Health) found that antisera to primate virus reverse transcriptase cross react with m antigen from human leukaemic cells,

and that human tumour cells contain an antigen cross-reactive with primate virus p30. Visual sightings of the mature Ctype particles in human tissue material, however, are rare. Non-enveloped particles, which contain 70S RNA and viral antigens (virosomes), and which actively incorporate labelled precursors in vitro, were described in the mitochondrial membrane of avian and murine tumours by J. Kara (Czechoslovak Academy of Sciences, Prague). These particles are more prominent in nonproducer tumours. Although the Spiegelman particles, but not the oncornavirus precursors identified in human cell lines by A. Bukrinskaya (Academy of Medical Sciences, Moscow), differ from virosomes in density, more than one worker went home anxious to isolate human mitochondria. Thus immunology's most useful contribution to cancer research at present may be in the identification of candidate human tumour viruses.

Many papers dealt with the immune response to tumour-specific antigens. On the clinical side, the meaning of cell mediated immunity in vitro was questioned in several papers, for example by M. Takasugi (University of California, Los Angeles), R. W. Baldwin (University of Nottingham), and F. J. Cummings (Brown University): the popular microcytotoxicity test can no longer be accepted as a measure of the response of the patient to his own tumour. Significant responses, if they occur at all, seem to do so most clearly with a restricted range of tumours which include bladder carcinoma, melanoma and sarcomas. Encouraging results were reported with 'immunotherapy'—both specific (with administration of tumour cells or membrane preparations) and nonspecific (with immunological adjuvants such as tubercle bacilli, where C. Maruyama (University of Tokyo) had particularly extensive data). In no case, however, was there decisive evidence of the involvement of an immune mechanism.

The use of immunological methods to detect and monitor tumour growth was concentrated mainly on carcinoembryonic antigen for carcinoma of the gut and alpha-foetoprotein (AFP) for hepatoma, with limited use of cellular immunity against cell lines in other types of cancer. Papers from Europe and the United States, all of which took a cautious line towards widespread population screening were \*dwarfed by one from the Coordinating Group for AFP (Pekin) which included data from a massive screen of 500,000 individuals. Those who wish to evaluate the test wait, therefore, to see whether the Chinese find the effort worthwhile.

Analysis of the immunological mechanisms which operate in animal tumour systems continues. The entire response

from induction to final killing can now be obtained in vitro, and the killer cells thus generated are highly active (J. C. Cerottini, Institute of Cancer Research, Lausanne). Efforts to characterise the antigens involved have now reached the stage reached 5-10 years ago with the major transplantation antigens. Virus-coded cell surface antigens are better characterised, and are available in some instances for in vitro manipulation with lymphocytes (G. R. Shellam, Imperial Cancer Research Fund, London). From the strictly immunological standpoint the furthest advance was reported by K. Rajewski (University of Koln), who has rendered antigens obsolete by elicitating a response with anti-idiotypic antibody, that is, with antibody directed against the antigen-combining region on the receptors of a clone of lymphoytes. Since his procedure stimulates T lymphocytes as well as B lymphocytes, it implies-subject to certain safeguards in interpretationthat T and B cells read from a common dictionary and share a common pool of receptors.

## Tumour viruses at Munich

from a Correspondent

TUMOUR virus meetings tend to have the same speakers, discuss the same subjects and send their participants away asking the same question: what do tumour viruses have to do with tumours? The clinical oncologist must helplessly face this question every day; meanwhile the basic oncologist faces the statistics that 60-90% of all known malignancies in man are caused by chemical carcinogens and that in no case so far have the candidate human cancer viruses been firmly identified as a cause of cancer in man. Their problems are intensified by the gap between experimental tumour research and clinical oncology. The tumour virus meeting held at the Max-Planck Institute for Biochemistry, Munich, from October 4 to 10 provided a good opportunity for communication between basic and clinical oncologists.

Dr R. Gallo (National Cancer Institute, Bethesda) discussed his concept of class I and class II viruses. He showed that the RNA genome of class I viruses (typical endogenous viruses such RD-114) hybridises virtually completely to DNA of normal uninfected cells. These virogenes may be incompletly expressed and their products, such as reverse transcriptase, can be associated with normal tissues. Class II viruses show much less hybridisation of their RNA genome to uninfected cell DNA. Members of this class, for example murine and feline leukaemia

and sarcoma viruses, can probably be transmitted horizontally.

Dr S. Spiegelman (Columbia University) who has little faith in inherited oncogenes, presented evidence which certainly speaks in favour of horizontal transmission. In his studies with identical twins he has found that the leukaemic twin contained particle-related DNA sequences that could not be detected in the leukocytes of his identical healthy sibling. Dr S. Dube (Max-Planck Institute, Göttingen) suggested that the mechanism of neoplasia could involve somatic hypermutation of vertically transmitted endogenous type C virogenes; if this hypothesis is correct, Dr Spiegelman would have seen no difference between leukaemic and healthy siblings if he had looked at germ line rather than leukocyte DNA.

Work on viral structural proteins is progressing rapidly. Drs Moenning and (Max-Planck Hunsmann Institute. Tübingen) described the purification and characterisation of the major coat protein p30 (molecular weight 30,000) and the major envelope glycoprotein gp71 of Friend erythroleukaemia virus. They showed that gp71 must reside in the surface knobs because treatment with bromelin removes both knobs and gp71. Dr M. Strand (Albert Einstein College, New York) reported that she finds an additional glycoprotein, gp69. Each of these proteins contains distinct interspecies antigenic determinants. The gp69/71 mixture can induce neutralising antibodies and thus seems to be potentially the most useful antigen for the preparation of vaccines. Dr F. Lilly (Albert Einstein College, New York) showed that gp69/71 is non-coordinately expressed with the p30 protein but appears to be partially linked to sarcoma genes. Dr Vaheri (University of Helsinki) using radioimmunoassay, showed that leukosis-free chicken cells expressed low concentration's of p30, but after infection the cellular concentration of p30 went up by 100-1,000 fold.

The nature of the viral genome was actively discussed. It is generally agreed that the genomic RNA is a 70S aggregate (molecular weight 10<sup>7</sup>) of several 30-35S (molecular weight  $\sim 3 \times 10^6$ ) subunits. Whether the subunits are identical or not has been a major issue. From the extensive genetic and biochemical evidence accumulated by Dr Vogt (University of Southern California, Los Angeles) and Dr Duesberg (University of California, Berkeley) it seems that the genome of Raus sarcoma virus is polyploid; that is, the subunits are identical. This supported by the independent findings of Drs Baluda (UCLA), Billeter (University of Zurich) and Dube.

Drs Wu and Gallo have been

studying compounds which affect virus replication and virus-induced transformation and which may therefore have clinical value. Dr Wu described the inhibitory effects of rifamycin SV derivatives on proviral formation and cordycepin on virus production at an early post-transcriptional step. Dr Chandra (Frankfurt) reported on the inhibitory effect of thio-polynucleotide homopolymers of cytidylic acid and uridylic acid.

#### Energy in agriculture

from N. R. McFarlane

At a meeting on October 21 held at the Society of Chemical Industry, chemical and mechanical aspects of energy utilisation in agriculture were discussed.

J. J. Landsberg of Long Ashton Research Station pointed out that the conversion of solar energy into useful biomass is an inefficient process. Man's efforts to increase useful biomass in agriculture moreover require considerable energy input in the form of fertilisers (Professor G. W. Cooke of Rothamsted Experimental Station). While other agricultural chemicals such as herbicides are less energetically demanding, requiring similar energy inputs to mechanical weeding methods (Dr M. B. Green of Imperial Chemical Industries) they are also less effective than fertilisers. The link between solar sources of energy and chemical energy was provided in the discussion of the direct use of coal, oil and gas for heating and mechanical operations on the farm and in the glasshouse (G. F. Sheard, Glasshouse Crops Research Institute).

The paper which created most discussion was that of Dr K. L. Blaxter of the Rowett Research Institute, who showed that known world reserves of energy (42×10<sup>15</sup> MJ) are equivalent to but two years of European sunshine! His Table 1 shows that only 11% of the total plant material harvested in the United Kingdom is 'farm gate' output, destined for human consumption. After processing this primary plant food

Table 1 Biological energetics of agriculture in the United Kingdom

ture in the United King	dom
(M	Energy IJ × 10° yr <sup>-1</sup> )
Solar radiation total incidence Gross primary production	610,000
harvested from plants Imports of foodstuffs	1,116
'Farm gate' output of crops 119	9
'Farm gate' output of	
Total farm output Edible food from crops 65	213
Edible food from animals 73 Total edible food	3
Energy required by the popular	137 ition 241

Table 2 Energy inputs for agriculture in the United Kingdom

	E	nergy
	$(MJ \times 1)$	$0^{-9} \text{ yr}^{-1}$
Fuel	•	144.5
Fertilisers and lime		128.1
Agrochemicals		1.2
Machinery		48.8
Processing of foodstuffs		2.1
Transport		15.7
	Total	340.4

yields 65 MJ×10° yr<sup>-1</sup> of energy for human consumption. 70% of the prime plant output is eaten by farm livestock—a reflection of the importance of the animal sector in British agriculture. Table 2 was also presented by Dr Blaxter to show the high energy inputs besides solar energy needed to maintain the agricultural output.

Both Professor E. W. Russell (University of Reading) and Dr R. J. Martin of Plant Protection Limited criticised short term economical evaluation of depleting natural resources. The message I received was that true economic value will not be placed upon such items as fossil fuels until it is too late. Even the use of conventional economic evaluation under such circumstances is somewhat suspect.

## Immunity and connective tissue

from A. C. Allison

THE Fifth Lepetit Colloquium on The Immunological Basis of Connective Tissue Disorders was held in Madrid on November 11-13. M. Mannik (University of Washington) described the self-association of IgG rheumatoid factors to form stable dimers in which one antibody binding site on each molecule reacts with an antigenic determinant on the Fc region of the other molecule. The calculated association constant for this dimerisation is remarkably high (10<sup>10</sup> l mol<sup>-1</sup>). In addition a concentration-dependent aggregation of dimers into tetramers. octamers and higher polymers is observed. The self association of rheumatoid factors may play a part in perpetuating immunologically mediated synovitis. Complexes of IgG and IgM antiglobulins that precipitate in the cold (cryoglobulins) are sometimes associated with renal disease (E. Franklin, New York University) and some patients with systemic lupus erythematosus (SLE) have been found to have idiotypic markers of monoclonal cryoglobulins in their renal lesions (H, Kunkel, Rockefeller University).

Several laboratories have been studying the antibodies against human T and B lymphocytes, some reactive only in the cold and others at 37° C, present in patients with SLE, rheumatoid arthritis (RA) and infectious mononucleosis. Messner (Albuquerque) reported the presence of cytotoxic antibodies against lymphocytes in the majority of family members of patients with SLE, irrespective of consanguinity. A virus or other transmissible agent may be involved.

F. Dixon (Scripps Clinic, La Jolla) emphasised the analogy between the role of endogenous virus antigens in mice and the appearance of SLE in humans. In all mouse strains virus-specific antigens are found, and in some strains complexes of these antigens and antibodies, as well as nucleic acids and antibodies, accumulate in the renal glomeruli.

R. Lerner (Scripps Clinic, La Jolla) presented evidence obtained in his

laboratory and that of E. Boyse in New York that a mouse thymocyte differentiation marker, GIX, is the same as a glycoprotein (gp70) in the virions of murine oncornaviruses. The oncornavirus genomes are integrated in the host genomes, and the expression of gp70 is under control of factors both genetic (manifestation on the membrane of thymocytes in GIX+ strains only) and environmental (manifestation only under the influence of the thymus). In G<sub>IX</sub> strains the antigen is present only in leukaemic cells. Thus the distinction between virus and host gene products becomes tenuous, and whether immune responses to them are regarded as autoimmune is a question of semantics. As in mice and chickens, all human cells seem to have in their genomes lengths of DNA hybridising with oncornavirus nucleic acid, and the number of copies of oncornavirus DNA is increased in SLE and leukaemia.

B. Pernis (Basel Institute for Immunology) pointed out that most human B lymphocytes have surface IgM as well as IgD, the proportion varying from cell to cell. These turn over at different rates: when they are lost after capping or protease treatment IgM is resynthesised much more rapidly than IgD. W. Hijmans (Institute for Experimental Gerontology, Rijswijk) reported that the only surface immunoglobulin of lymphocytes in young human foetuses is IgM, which invalidates the view that IgD is the first to appear.

H. Muller-Eberhard (Scripps Clinic, La Jolla) described the amino-acid sequence of the complement component C3. The cleavage product C3a requires a high  $\alpha$ -helical structure for activity. When C-terminal arginine is removed from C3a, the product binds to mast cell receptors and norepinephrine receptors of blood vessels and blocks their activation. K. Austen (Harvard) emphasised the symmetry of the steps involved in activation of complement by the classical and alternate pathways. Both pathways are activated in the joint fluids of rheumatoid arthritic patients. Austen concludes that the alternative pathway is activated ab initio but another explanation, that there is feedback activation from the classical pathway, is difficult to exclude.

J. Natvig (Institute of Immunology and Rheumatology, Oslo) reviewed recent observations on the heterogeneity of amyloid. A non-immunoglobulin AA protein from tissues of different patients shows identity over most of the 76 residues but some variation in the C-terminal region. Levels of the related serum component (SAA) are increased in sera of patients with rheumatoid arthritis and in old people. An immunoglobulin isolated from amyloid shows homology with  $\lambda$ -chains over the first 45 residues and seems to represent a new  $\lambda$ -variable subgroup.

L. Glynn (Kennedy Institute, London) discussed his model of persistent monoarticular arthritis following intra-articular injection of antigen into previously immunised animals. Complexes of antigen and antibody are found in menisci, but it is not clear whether these have any pathogenetic role. Release of hydrolytic enzymes from mononuclear phagocytes exposed to immune complexes or products of activated lymphocytes, discussed by A. Allison (Clinical Research Centre, Harrow), may be involved in joint damage in rheumatoid arthritis. There is still no convincing evidence of any causal role of an infectious agent in rheumatoid arthritis.

#### Immunopathology of parasitic infection

F.E.G.C. REPLIES to the responses in Matters Arising last week (252, 509; 1974) to his News and Views article (246, 187; 1973).

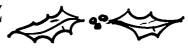
My statement that "most parasites are capable of evoking immune responses in their hosts but these are seldom effective in eliminating the infection" implies that some parasites do mount effective immune responses. Professor Urquhart and Dr Miller both refer to lungworm disease of cattle thus stressing the comparative rarity of the latter situation. It is true that there are well defined immune responses that eventually result in some degree of protective immunity against parasitic infections. But it is equally true that the actual elimination of the parasites as a result of an immunological response is not commonplace. The major parasitic diseases of man (malaria, sleeping sickness, Chagas' disease, American leishmaniasis, schistosomiasis and filariasis) are characterised by infections that last months or years. To most people this indicates a failure of the immune response to eliminate the infection. The immune response may ameliorate the disease symptoms but transmission of the parasite still occurs. Domesticated animals are more fortunate than man in being able to mount effective immune responses but in piroplasmosis and fascioliasis these responses are not very effective. It must also be remembered that helminth infections may diminish as a result of the natural death of the worms and not an immune response. This aspect of recovery from infection has been

little studied.

My statement that "the possibilities of developing useful methods of immunisation [against parasitic diseases] fade further and further into the distance" does not imply that immunisation is always going to be impossible. Vaccination against cattle lungworm, canine hookworm and certain kinds of piroplasmosis has been achieved but the vaccines used, irradiated larvae or infected blood, are unacceptable in the field of human medicine. With the development of a vaccine against cattle lungworm nearly twenty years ago, it was felt by many workers that the solution to the problem of vaccination against parasitic infections was just round the corner. This early optimism has not been justified. As more and more is discovered about immunity to parasitic infections further complications are uncovered. The development of vaccines against many parasitic diseases may not be possible before the diseases are eliminated by drugs or public health measures. Trichinellosis is a case in point. There have been encouraging reports of possible immunisation procedures against Trichinella but its decline in the United States has been brought about by legislation designed to prevent swine fever. Studies on the immunological responses to parasites have been amply rewarded by discoveries in pure immunology, the development of immunodiagnostic techniques and an understanding of many immunopathological conditions. Vaccines will also be developed as a result of these studies—but not for many years to come.



Christmas quiz



- 1. Who says we should harness eccentric buoys?
- 2. What is the next number in the series 1, 2, 3, 5, 8, 13, 21, 34, 55 ... and in what way is this series linked both to Pisa and to leaves?
- 3. What professor of chemistry wrote an opera whose most famous tune was, many years later, used in the musical 'Kismet'?
- 4. She wanted his next book dedicated to her and indeed it was—but instead of being a story of wide popular acclaim, it was an elementary treatise on determinants. Who was she?
- 5. Who has an answer to Logical Positivism in the 'family Four Position Base'?
- 6. Appearing twice a year, they are common in India, China and Japan but are probably indigenous to western Asia. In ancient Egypt one variety was accorded some degree of divinity and is featured on monuments of that period. If you had one what would you do with it?
- 7. He studied in Vienna and by the age of 26 had become Professor of Mathematics at Gratz University. Four years later he moved to the Chair of Physics in Vienna, and it is as a physicist and psychologist that he is remembered most. Towards the end of his life he became a member of the Austrian house of Peers. The rather one sided view from one of his philosophical works was featured briefly in a recent edition of *Nature*; what was it and who was he?
- 8. What was the concept that was first suggested publicly in an address in Frankfurt am Main in January 1912; and who delivered the address?
  - 9. Where and when did the largest single oil spill occur?
- 10. What is the most commonly used tricyclic anti-depressant and what are the main effects of such compounds?
- 11. What is dendrochronology and what importance does it have in art?
  - 12. Of what biochemicals are these the empirical

- formulae: (a)  $C_{35}H_{28}MgN_4O_5$ ; (b)  $C_{63}H_{88}CoN_{14}O_{14}P$ ; (c)  $C_{34}H_{32}ClFeN_4O_4$ ?
- 13. Name the group which in 1974 officially wanted restriction restricted?
- 14. By what discovery in 1974 was St Bartholomew's Hospital specifically linked to a gene deletion?
- 15. Which enzymes are deficient in: (a) Fabrys disease; (b) Sandhoffs disease; (c) Pompes disease?
  - 16. Who saw fruit in a padded cell?
- 17. From where, precisely, was Marconi's first transatlantic radio transmission made?
- 18. How many optical telescopes bigger than 100 inches are in operation or under construction in the Southern Hemisphere and where are they?
- 19. Some useful quotations for authors to know—but who said them first?
- (a) Accuse not Nature, she hath done her part ...
- (b) can't be Nature . . . not sense . . .
- (c) except the blind forces of Nature, nothing moves in this world ...
- (d) The fault was Nature's fault not thine ...
- (e) What's a' the jargon of your schools; Your Latin names for horns and stools; If honest Nature made you fools.
- (f) Thrice happy he who not mistook Hath read in Nature's mystic book ...
- (g) to be constant, in Nature were inconstancy . . .
- (h) Nature is always wise in every part . . :
- (i) Nature abhors imperfect work . . .
- (i) But in them Nature's copy not eterne . . .
- (k) Nature knows a thing or two . . .
  - 20. Who wrote:

Nature and Nature's laws lay hid in night: God said let Newton be! and all was light and who replied with what?

21. For what work did the following win Nobel Prizes: D. Bovet (1957); J. Heyrovsky (1959); G. von Bekesy (1961); G. Wilkinson (1973)?

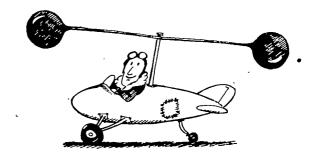
See page 622 for answers.

### Spinning into space?

RECENT publicity about possible 'antigravity' devices which seem to defy Newton with the aid of a spinning top or two encourages us to offer for seasonable speculation the following question, taken from the 1969 Christmas Examination for Junior and Senior Honours in the Natural Philosophy Department of the University of Aberdeen. There is no prize for any correct answer, and no answer is presented in the pages of this issue of *Nature*.

"There is currently an application for a patent for an antigravity device based on the following argument:

'A particle at the Earth's surface has a critical horizontal velocity  $\nu$  such that this velocity will cause it to orbit the Earth in a circle. If this velocity is exceeded, the particle will tend into an orbit at a greater mean distance from the Earth's centre. The actual direction of the velocity in a horizontal plane makes no difference to the foregoing conclusion. Now such a horizontal velocity can in principle be achieved by spinning two particles joined by a horizontal bar as in a dumbell. Each particle can thus be



made to have a velocity V which is greater than v, and so they should jointly rise. The proposed method of defying gravity therefore consists in spinning a dumbell fast enough.

What is the flaw in the argument, and how would you try to persuade the inventor (an imaginative engineer who might not be convinced by an argument based on the motion of a common centre of gravity) that he would be foolish to waste money on pursuing the patent?"

### Science journalists come of age in Pisces

John Gribbin\*

It seems that more science journalists are born under Pisces than any other sign.

WINDSOR<sup>1</sup> has pointed out that the astrological belief in the importance of the 'Sun sign' as an influence on personality seems to be reflected in the distribution of birth dates of American biologists. In particular, he found that molecular biologists tend to be born under Aries. I have now compared the birthdays of science journalists found in the London office of *Nature* with those of all staff members found in that office. The birthdays were obtained by personal questioning during August, 1974, and the appropriate Sun signs were assigned.

More science journalists were born under the sign of Pisces than any other sign (Fig. 1), and relatively few (zero) members of the *Nature* staff were born under Aries, Taurus or Gemini. In spite of the small size of the sample, the result is significant as indicated by Lynden-Bell's half-power test. This test uses the criterion that in statistics, all numbers go as the square root (D. Lynden-Bell, unpublished).

Taking the data for science journalists alone, there are eight Pisces and eight others (including one "no comment"). The most densely populated Sun sign other than Pisces is Leo, with just two members—less than even the half-power of the Pisces population.;

It may also be significant that of the Leo population one (E) is the Editor of *Nature* and the other (DE) is Deputy Editor.

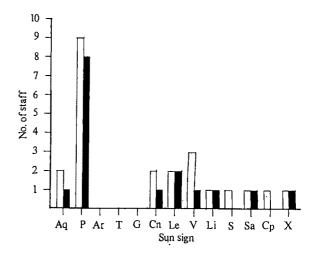


Fig. 1 Frequencies of Sun signs of *Nature* staff. Open histogram, all staff; solid histogram, science journalists only; X, birthday not revealed ("no comment").

While these results are suggestive, further observations of a larger sample are needed before any firm conclusions can be drawn.

\* Nature, London

# Ambisonic reproduction of directionality in surround-sound systems

P. B. Fellgett\*

In both the technology and the aesthetics of extending high fidelity reproduction to surround-sound, reproduction of natural ambience is crucial. The 'quadraphonic' attempt to reproduce four stereoblended tracks, derived from multi-microphone mix-down, cannot provide this. Complete spherical directionality can however be encoded on to a minimum of two audio channels to produce acoustically acceptable surround-sound systems. Limitations are set both by the number of available loudspeakers and by the number of channels.

The history of representational art has many examples of what people of a particular age and culture were unable to perceive, and therefore to reproduce; for instance the inability of the dynastic Egyptians to perceive that the full-face eye is not seen in a profile figure. The reproduction of sound follows a similar pattern. In the early development of the phonograph and of 'wireless' the listener was so satisfied with the novelty of hearing the human voice or music issue from a machine that he demanded nothing else. Indeed there was often little conscious

appreciation that anything was lacking in fidelity of reproduction; how else could Sir Arthur Conan Doyle suppose in *The Adventure of the Mazarin Stone* that Sherlock Holmes could deceive a sophisticated villain into mistaking a 'modern gramophone' of 1927 for real violin playing, even in the next room. As late as the 1930s, many listeners could see little short of perfection in the commercial 'radiogram'.

As the sense of wonder wore off, however, a more critical attitude filtered down from audio engineers to the buying public, and the search begån for high fidelity reproduction.

During and immediately after the Second World War, domestic reproduction of sound depended on the 78-r.p.m.

<sup>&</sup>lt;sup>1</sup> Windsor, D. A., Nature, 248, 788 (1974).

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shellac disk and a.m. broadcasting. Now with the vinyl disk and f.m. broadcasting, high fidelity reproduction has reached the stage at which the best equipment can make a chamber group sound (to me) like the real thing; but this degree of realism in the reproduction of an orchestra still eludes us. Careful introspection suggests that even this judgement is too lenient, and that we are 'listening-through' major defects which have always been inseparable from reproduced sound, and which therefore we take for granted.

One aspect that is obviously lacking is directionality. Monophonic reproduction gave no explicit directional information, even when reproduced from more than one loudspeaker. In stereophonic reproduction, sound reaches the listener only from the forward direction, whereas in the concert hall the listener is bathed in reverberant sound coming from all directions. In addition, stereo works by feeding common signal-components to a pair of loudspeakers with relative amplitude determined by the position which the illusory sound source is to occupy. It is easy to show that the perception of stereo-imagery is both physically artificial and aesthetically untrue to life; it has to be learnt and some 10% of the population are unable to do this. Moreover, although skilful recording can give some illusion of depth, the acoustic images are essentially strung out along a line joining the two loudspeakers, giving an effect that has been described as a cardboard cut-out orchestra.

### Role of directionality

From the antiphonal tradition through to the school of St Mark's and the baroque, directionality played a large and explicit part in western music. In classical and romantic orchestral music this explicit component is largely abandoned, some composers even preferring a completely blended sound, but nevertheless directionality is important implicitly. A composer necessarily writes with subconscious experience of the relationship between the sections of an orchestra, or between the different departments of an organ and the relationship of these to the choir; it has been said that the works of the Bach family cannot be fully appreciated except on an organ constructed on the Werkprinzip.

Some conductors pay considerable attention to the disposition of the sections of the orchestra, and these relationships should be heard in reproduction. There is little need, however, to pinpoint individual instruments, which indeed is seldom possible in the concert hall. What is important is that each voice in the musical texture should be labelled with an acoustic ambience characteristic of its place of origin. The ability to follow different voices in the musical texture, despite substantial differences in level, is very much bound up with this labelling, as indeed is the appreciation of musical timbre itself.

All these aspects relate to the acoustical reverberance and ambience of the concert hall. If it is worth spending thousands of pounds on the acoustics of a concert hall, then this should be heard in reproduction.

It follows that the highest fidelity in sound reproduction requires that the directionality of sound should mimic both the direct and reverberant sound of the concert hall. This is not just a matter of a vague splash of delayed echoes, but of a relationship between directionality and time-delay which gives specific information; this information is what we shall mean strictly by ambience. Systems capable of reproducing it will be called 'ambisonic'.

### 'Quadraphonic' attempt

In the recording industry it is customary to mix-down multimicrophone recordings onto four-track master tape in which signals are often stereo-blended between pairs of channels. The present upsurge of interest in surround-sound was in some measure triggered by engineers and producers playing back such four-track material directly into four amplifiers and loudspeakers distributed approximately in a square near to the corners of the monitor room.

Mixed-down tapes are unfortunately highly contrived and may have only the sketchiest relationship to natural sound. Moreover the assumption was that directionality should be encoded by pair-wise blending between channels associated with adjacent corner positions. This is a sub-optimal method of encoding, making less than full use of the information capacity of the available channels. Further, in order to achieve this coding using directional microphones it would be necessary for the polar response to have the highly improbable form of a half-cycle cosine wave over one sector of 180°, and zero response over the remaining 180°.

There are also severe restrictions in playback. If four loudspeakers are disposed about a listener, at least one pair must subtend an angle of 90° or greater at that listener. It is well established, however, that stereo blending does not work well when the angular subtense of the line joining the speakers exceeds about 60°. Moreover, stereo imagery works best over the 90° sector in front of the listener, worse at the rear, and worse still at the two sides. The attempt to extend the principle of stereo blending from the front wall to the other three sides of the listening room therefore not only retains all the limitations of stereo itself (outlined earlier) but is necessarily rather poor even judged as stereo.

The above approach is what is usually meant by the term 'quadraphony', although usage is by no means uniform. The name itself is a canard in at least two ways. First, it is a Greek—Latin hybrid, and ought to be called quadrisonic. The second is the identification, by implication, of surround-sound with the need to have four channels. In fact, as we shall see, surround-sound can be realised with as few as two channels. The false assumption that surround-sound is synonymous with four-channel then introduces the need to find distinctive names for two- and four-channel surround-sound systems, which were therefore called 'matrixed four-channel' and 'discrete four-channel', respectively. To prevent confusion I shall not use these jargon terms, and words such as four-channel and matrix will be used simply in their ordinary English and technical senses.

#### More systematic approach

Any system that is to reproduce specific ambience information must embody the following (Fig. 1):

- (a) transduction of the original sound field, including its directionality and acoustic ambience;
- (b) encoding the resultant composite signal, including information about directionality, on to the available communication channels;
- (c) transmitting the resultant information to the listener, including recording and storage of this information where appropriate;
- (d) decoding the channel-signals into a form suitable for feeding to a plurality of loudspeakers;

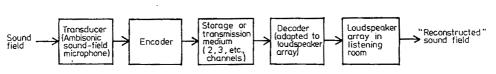


Fig. 1 Basic features of ambisonic reproduction.

(e) radiation from the loudspeakers of sounds which interact in the listening space so as to reproduce, as far as possible, a simulacrum of the original sound field.

Our present systematic understanding of the requirements and possibilities of these steps owes much to the work of Gerzon<sup>2</sup>, particularly his analysis of the theory of encoding and of psycho-acoustic criteria in the reproduction of directionality, much of which is not yet published. The sections which next follow will discuss some of the salient problems in greater detail.

### Sound-field microphones

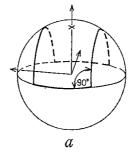
It is first necessary to characterise the sound-field at a given place in the room where the performance is taking place. The sound field at a point can conveniently be characterised in terms of spherical harmonics representing the sound field pressure and its derivatives. The number of spherical harmonics up to and including various orders n is shown in Table 1. From this it is seen that the number of independent signals, each representing a spherical harmonic, is of the form  $(n + 1)^2$ . The 0th order harmonic is equivalent to the sound-field pressure alone, and gives simply mono without directional information. The three spherical harmonics of order 1 are equivalent to the three cartesian components of particle velocity  $v_x$ ,  $v_y$  and  $v_z$ . Higher orders of spherical harmonics represent non-redundant combinations of higher gradients; they are of potential interest for the future but need not be considered for the purpose of current developments.

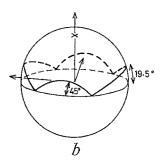
In order to deal properly with indirect sound, which may of course arrive from any direction, a sound-field microphone must encode in a deliberately designed manner any sound reaching it, including sounds having a vertical component of travel. It can be shown that microphones which violate this condition necessarily introduce undesirable coloration into the reproduction of reverberant sounds, even when the vertical information is deliberately suppressed in subsequent processing. From this requirement, having regard to Table 1, it follows that the number of independent signals generated by a microphone should correspond to the square of the natural numbers;  $(n+1)^2 = 1$  giving monophonic reproduction, and  $(n+1)^2 = 4$  the minimum uncoloured reproduction which includes directionality.

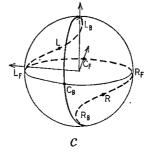
	Table 1 Spherical harmonics				
Order 0 1 2 n	No. of harmonics $ \begin{array}{c} 1 \\ 3 \\ 5 \\ 2n+1 \end{array} $	Cumulative total $ \begin{array}{c} 1\\4\\9\\(n+1)^2 \end{array} $			

Gerzon has shown that particular polyhedral arrangements of microphone capsules can fulfil the relevant requirements. The simplest of these places the capsules at the face centres of the regular tetrahedron. The spacing of the capsules should be kept as small as possible, but as it cannot practicably be small compared with the wavelength at higher audio frequencies, correction circuits are needed to compensate for the resultant disturbance between the pressure and velocity responses of the composite microphone. There are some advantages in using a larger polyhedral array to generate a  $(n+1)^2 = 4$  signal, and these are likely to be explored in the future.

The earliest ambisonic experiments were made using an improvised tetrahedral array of ordinary cardioid-response microphones. This arrangement, however, gives rise to considerable mutual interference, and it is impossible to obtain a sufficiently close spacing. Use of microphones in undesigned non-polyhedral arrays, or with spacings of 30 cm or more, or using replay direct into loudspeakers without pressure-velocity compensation, all give markedly sub-optimal results.







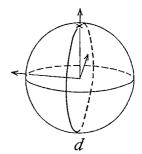


Fig. 2 Some representative horizontal pan-loci. a, Sansui 'QS'; b, closer approximation to great-circle locus consistent with four-channel master tapes (not in commercial use); c, CBS 'SQ'. Note cusps and left-right asymmetry due to choice of front-sector mapping and limitations of encoding from four pair-wise blended channels. C<sub>F</sub>, Centre front; C<sub>B</sub>, centre back; L, full left; R, full right; L<sub>F</sub>, left front; and so on; d, a great-circle locus consistent with ambisonic encoding.

It is desirable to subject the raw signals from the sound-field microphone to a  $4 \times 4$  matrix transformation into a form, call B-format, in which they have reduced sensitivity to small phase or amplitude errors in transmission or recording.

#### Encoding

The problem of encoding of directional information may be stated either in the form of how to make best use of a given number of available channels of communication, or of asking how many channels are necessary and sufficient for a given purpose.

Direction of travel can be characterised by the azimuth angle  $\theta$  and the altitude angle  $(\pi/2-\phi)$ . Both parameters can be encoded on to only two channels carrying signals A and B for example as

$$|\mathbf{B}|/|\mathbf{A}| = \tan(\varphi/2) \tag{1}$$

$$LB - LA = 0 \tag{2}$$

There are of course many other informationally-equivalent forms of these equations. In equation (1),  $\tan(\phi/2)$  is taken in order to avoid the 180° ambiguity that would result from using  $\tan \phi$ .

Equations (1) and (2) can equivalently be regarded as defining polar angles  $\theta$  and  $\phi$  (not necessarily identical with azimuth and altitude) which characterise the relative amplitude and relative phase of signals encoded on to two channels. Any given encoding can then be represented graphically by a point on a sphere, known as the Poincaré or energy sphere³. This graphical representation is extremely useful in enabling the properties of proposed coding schemes to be understood, and it possesses a number of useful mathematical properties. The path on the energy sphere described by the representative point as the azimuth explores the horizontal circle is known as the horizontal pan locus; some representative examples are illustrated in Fig. 2.

There are several advantages in making this locus a great circle, and little or nothing to be gained by deviating from this form. Gerzon has shown that matrixing four pairwise-blended channels onto two channels, as in so-called quadraphony, necessarily gives a pan-locus consisting of four circular arcs intersecting so as to include a total angle of  $\pi$  rad at their corners; it therefore cannot give a great circle locus.

Encodings on to three or more channels can be represented similarly by points on hyper-spheres of appropriate dimensionality, but this is somewhat less useful as an aid to intuition because of the difficulty of graphical representation.

Equations (1) and (2) show that directionality can be completely encoded on to only two channels in the 'direction finding' sense, that is, any direction of arrival can be represented unambiguously. This is evidently necessary, but it is not sufficient, to ensure satisfactory performance of the overall ambisonic system, because it does not of itself ensure that the signal channels can be decoded in the required manner.

### Decoding and psycho-acoustic criteria

An exact replica of the original sound field, in the whole region occupied by the listener's head and up to the highest audible frequencies, would require many thousands of audio channels of communication and is quite impracticable. The closeness of approximation that can be achieved depends both on the number of channels and on the number of loudspeakers that are available.

At frequencies low enough for the human head to be small compared with the wavelength of sound, it is indeed possible to reproduce physically the original sound field. At higher frequencies this is no longer possible, and the best that can be done is to fulfil as many as possible of the psycho-acoustic criteria whereby sound directions are localised. These criteria, in relation to practicable systems, have been established particularly by the work of Gerzon already referred to.

Ambisonic reproduction in which vertical directional information is preserved may be called periphonic, and ambisonic reproduction with only horizontal information pantophonic. Pantophonic reproduction geometrically requires a minimum of three loudspeakers, since the triangle is the minimal figure able to enclose space in a plane. Similarly a minimum of four loudspeakers are geometrically necessary to surround the listener in three dimensions and give periphonic reproduction.

To satisfy the psycho-acoustic criteria sufficiently well, however, the practical minimum is four loudspeakers for pantophonic reproduction and six for periphony. One of the effects of using too few loudspeakers is that the loudspeakers themselves can be heard obtrusively as separate sources in addition to any ambisonic impression. This is closely associated with the 'detent effect' whereby as an actual source of sound moves its reproduced image seems to cling to each loudspeaker before jumping relatively rapidly to the next.

The limitations set by the number of loudspeakers interact with those set by the number of available channels. Qualitatively this is as expected, since the available number of loudspeakers limits the number of constraints available to control the sound field in the listening room, while the number of channels places restrictions on the extent to which these control constraints are independent. If four loudspeakers is taken as the practicable minimum for pantophonic reproduction, this is also in most people's minds at present the economic maximum. It can be shown that the capabilities of four loudspeakers in a planar array can be fully exploited using just three independent channels of audio information. By a happy coincidence, the current system of f.m. stereo broadcasting has sufficient bandwidth to transmit three audio channels. Interestingly, if the attempt is made to feed four loudspeakers from four channels in a non-redundant manner, the result is to enhance the detent effect. It can be shown that similarly four channels are sufficient to feed the minimal number of six loudspeakers required for periphonic reproduction. In general, the number of loudspeakers should

exceed the number of channels, so that it always pays to interpose a decoder between the communication channels and loud-speakers.

The practical outcome is that a two-channel four-loudspeaker pantophonic system can give good directional localisation with fulfilment of the salient psycho-acoustic criteria over a larger area than two-speaker stereo. Under favourable conditions, in surroundings that are acoustically neither too live nor too dead, the area of satisfactory and relaxed listening can fill most of an ordinary domestic room. The chief defect is a relative phase shift of 180° around the azimuth circle, unavoidable in any two-channel system, and the best that can be done is to distribute it in an optimally compromised manner. Current ambisonic decoders provide adjustments to compensate for spherical-wave effects of finite loudspeaker distance, and for non-square loudspeaker layouts.

The use of three channels enables the phase difficulty to be removed, and gives some improvement in the accuracy and stability of directional location. The next step in improving pantophonic reproduction is, as already indicated, to add additional loudspeakers rather than additional channels (which can actually make the results worse). Periphonic reproduction is, in the opinion of some who have heard it, at least as great an advance on pantophonic as the latter is on stereo, or stereo on mono. Three-channel periphonic systems may be possible with compromises not dissimilar to those involved in two-channel pantophonic reproduction. The minimum requirement of six loudspeakers in a non-planar array inhibits commercial interest at present, although periphony may have considerable interest for the future.

Beyond the  $(n+1)^2 = 4$  periphonic system, the next basis is  $(n+1)^2 = 9$ , which is also very much a matter for the future.

### Monophonic and stereophonic compatibility

The introduction of a new system always involves problems of compatibility with what went before. Ambisonic compatibility is therefore essentially a matter of the compatibility of its two-channel realisation with the existing two-channel mono and stereo formats.

Complete aesthetic compatibility is never possible between an old system and a new one providing additional information, as for example between stereo and mono or between colour and monochrome television. Technical compatibility depends on which out of a gamut of informationally equivalent ambisonic codings is employed. For ambisonic playback, these codings are equivalent because there is freedom to design the decoder to match the encoding. Ambisonic encoding can easily be made compatible with either stereo or mono, but not both simultaneously. A compromise is necessary, and the basic cause of this is not any fault in ambisonic encoding, but in the historical accident that the apparently obvious stereo and mono encodings concealed an inherent mutual incompatibility. Essentially, a balance must be struck between some attenuation of rear sources and some phase shift between the two loudspeakers in stereo playback; fortunately this can be done in a fairly harmless way.

Ambisonics is still in the experimental stage. It can be realised using two, three or more channels. The two-channel version has particular interest because of the wide availability of two-channel recording and broadcasting media developed originally for stereo use, and the three-channel version has particular interest in relation to f.m. broadcasting. Ambisonics is a complete system from the pick-up of the original sound field through encoding and decoding to the reconstruction of an approximation to the original sound-field in the listener's home. It therefore requires the simultaneous availability of both hardware and software; that is, of suitable decoders and of recordings or broadcasts on which to use them. None of these are commercially available at the present time, but may become so before very long.

Within the limitations of this article the attempt has been made to outline the basic principles of ambisonics with a minimum

of purely technical detail. Ambisonics is not so much a single system as a family of realisations having in common the attempt to state clear aesthetic and technological aims, to adjust these aims to what is physically and mathematically possible, and then to implement a properly engineered system to fulfil the objectives.

The development has been performed principally by M. Gerzon, J. S. Wright and the author with the help and support of the National Research Development Corporation. The experimental equipment was built mainly in the Research Unit for Instrument Physics of the University of Reading. The help of numerous academic and industrial colleagues is also gratefully acknowledged.

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### Statistical physics, particle masses and the cosmological coincidences

J. K. Lawrence\* & G. Szamosi†

A new look at some familiar 'cosmological coincidences' provides a means of 'predicting' appropriate mass values for the elementary particles.

For many years, cosmologists have been intrigued by possibly coincidental relationships between physical constants of the microscopic and cosmic domains1. Examples of these are the approximate equality of such large dimensionless numbers as the ratio of the electromagnetic to the gravitational interaction strengths of particles, the ratio of the size of the Universe to the size of an elementary particle and the square root of the number of particles in the Universe. There has been controversy, not only over the possible meaning of these 'cosmological coincidences', but also over the existence of any meaning at all. Here we would like to show how, by means of simple assumptions about the nature of physical parameters and of the Universe, the cosmological coincidences may be derived in a logically coherent scheme. Equally important, this procedure yields a very reasonable limit for the mass of an elementary particle expressed in terms of fundamental constants. An extension of the procedure leads also to an expression for the fundamental electric charge.

The usual view of elementary particles is that similar particles are absolutely identical—that they have absolutely identical masses, electrical or nuclear interaction strengths, magnetic moments, and so on. A less restrictive view, and one perhaps more in keeping with our views of the physics of the early Universe, might be that the properties of elementary particles are determined stochastically. Thus, some physical parameters of a set of elementary particles, particularly their masses, might not be absolutely identical, but rather form statistical distributions about average values, which depend upon the overall thermal properties of the Universe. According to the usual principles of statistics, the standard deviation of the equilibrium mass would be

$$\delta m/m \approx 1/\sqrt{n} \tag{1}$$

where n is the number of particles in the Universe. Whether n is considered to be the total number of particles in the Universe or the number of a given species, such as electrons or nucleons, is not important within the accuracy considered here. In this same spirit, we regard the (average) masses of all elementary particles to be essentially equal.

#### Variations of mass

We can now further restrict the mass fluctuations, using an anthropocentric argument similar to ones used in the past by

Dicke<sup>2</sup> and Carter in unpublished work. The existence of human life and awareness requires that the Pauli exclusion principle act over cosmological times. The nuclei of the various elements from which we are constructed must have existed for at least the age of the Solar System, and the structure of these nuclei depends on the Pauli principle, which depends on the indistinguishability of protons and of neutrons. If the masses of these nucleons differed by a detectable amount, the particles would no longer be indistinguishable and the Pauli principle would break down. According to quantum mechanics, in the age T of the Universe (or of the Solar System) it is possible to detect a mass difference  $\hbar/c^2T$ . Thus we conclude that the mass spread of elementary particles must be less than this critical value. Then,

$$\delta m \approx \hbar/K_1 c^2 T \tag{2}$$

where  $K_1 > 1$  is a dimensionless constant.

We next make the assumption that the Universe, at least approximately, is just gravitationally bound. This allows us to relate the gravitational constant G, the mass M, the radius Rand the age of the Universe by

$$R \approx cT \approx GM/c^2 \approx Gmn/c^2$$
 (3)

This relationship, sometimes connected with Mach's principle, is roughly compatible with observations and is widely assumed by cosmologists.

It is now possible to combine equations (1), (2) and (3) to obtain an expression for the mass of an elementary particle in terms of fundamental constants: .

$$m \approx (\hbar^2/K_1GcT)^{1/3} \tag{4}$$

Taking  $T \approx 10^{10}$  yr gives the numerical relation

$$m \approx K_1^{-1/3} \times 10^{-25} \,\mathrm{g}$$
 (5)

This gives an upper limit for elementary particle masses which is consistent with the existence of human life in the Universe:

$$m \lesssim 10^{-25} \text{ g} \tag{6}$$

A wide range of values of  $K_1$ , covering many orders of magnitude, will give values of m lying within the range of elementary particle rest masses ( $\sim 10^{-27}$  g to  $\sim 10^{-24}$  g). A recent study by Reines and Sobel3 indicates that the lifetime of the Pauli principle for inner shell electrons in iodine may be  $\sim T \times 10^{10}$  yr. Since the breakdown rate would be proportional to the square

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of the perturbation  $\delta m$ , this result implies a value  $K_1 \approx 10^5$  for these particles. This, when substituted into equation (5) implies  $m \simeq 10^{-27}$  g, the electron mass. It would be of great interest to measure the analogous Pauli breakdown rate for heavier particles, such as nuclear protons or neutrons. A value  $K_1 \approx 1$  also gives a mass well within the range of known particle masses, and for convenience we will now adopt this value. It should be kept in mind that both the theory presented here and the corresponding observational results are very rough. Thus large changes in the value of  $K_1$  will have no fundamental effect on any of the following equations.

### Cosmological coincidence

Apparently, Weinberg4 first noted the existence of equation (4) as a numerical coincidence and discussed its possible importance as a clue for a connection between microphysics and the Universe as a whole. The above considerations appear to give a possible rationale for its existence.

If we use  $K_1 \approx 1$  in equation (5), we find  $n \approx 10^{80}$ , which is compatible with present data, and  $\delta m \approx 10^{-65}$  g. By eliminating G from equations (3) and (4), we obtain

$$R/(\hbar/mc) \approx \sqrt{n}$$
 (7)

Elimination of R yields

$$m \approx m_*/n^{1/4} \tag{8}$$

with  $m_{\star} = (\hbar c/G)^{1/2}$ , the Planck mass. Also one finds the gravitational fine structure constant

$$\hbar c/Gm^2 = (e^2/Gm^2)(1/\alpha) \approx \sqrt{n}$$
 (9)

Equations (7) to (9) have often been noted in the literature as the 'cosmological coincidences.'

It is of further interest to consider the effect induced on particle self-energies by the variation  $\delta m$ . By requiring the induced variations in self-energy to remain too small for detection in the age of the Universe, as in the argument leading to equation (2), we obtain constraints on the values of the interaction coupling constants. We consider the formula for the lowest order electromagnetic self-energy of an electron given by Salam et al.5 where the ultraviolet divergence has been removed by inclusion of gravitational self-energy:

$$E \approx (me^2c/\hbar) (\ln n) \tag{10}$$

Requiring the variation induced in this by  $\delta m$  to be undetectable gives for the fine structure constant

$$e^2/\hbar c \approx (K_1/K_2)/(\ln n) \tag{11}$$

Where  $K_2 > 1$  is another dimensionless constant. This result is roughly correct if  $K_2 \approx K_1$ .

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### Lightning in astronomy

E. W. Crew\*

The author suggests that evidence for lightning on a grand scale in astronomy is most convincing. It might explain stellar flares, cosmic jets, quasars, galactic evolution, and much more. In this model, electrical charges accumulate in apparently highly conducting atmospheres.

Most of the characteristics of violent and sudden events1 in astronomy can be explained simply in terms of well-established laws of physics if electrical discharges, similar to lightning, occur in the atmosphere of stars and galaxies. This entails the formation and separation of positive and negative charges of sufficient energy to produce electrical breakdown in gases which generally have far greater conductivity than the relatively cold and dense atmosphere of the Earth. Careful examination of the evidence for electrical discharges in astronomy shows, however, that these almost certainly do occur and therefore charging does take place. This was first suggested by C. E. R. Bruce in 1941, following extensive research on laboratory electrical discharges and lightning as a member of the Electrical Research Association.

I do not claim to conform with Bruce's views, but this article should give an indication of the scope and value of the work. Details have been published in many letters in scientific and technical journals, but the description of his theory as a whole, essential for understanding these letters, has had rather limited distribution, mainly in a report with full references<sup>2</sup>, a chapter in a popular science book<sup>3</sup> and a conference publication4.

The study of terrestrial lightning is full of uncertainties because of its unpredictable location and brief duration<sup>5</sup>. The immensely more powerful and prolonged discharges in astro· nomy should help in the understanding of the processes in our atmosphere. The key to many astronomical events is the typical characteristic of an atmospheric discharge: a very rapid rise to maximum current, a steep decline, then a gradual fall over a relatively long period, with in some cases a secondary smaller rise of current, such as occurs in flare stars.

### Solar characteristics

If electrical charges accumulate in the Sun's atmosphere until sudden breakdown occurs, the discharges and induced currents will produce all the high temperatures and ionised conditions which seem to most astronomers to prevent electrical charging. It is therefore advisable to examine more closely how charging could occur, instead of rejecting it on the basis of superficial appearances. The generation of charges is chiefly by the friction of glancing collisions between solid particles or liquid drops, much as in the Earth's atmosphere. Other processes such as splitting during solidifying and unequal size disintegration of drops with induced charges are probably also significant. Particles of high melting point metallic materials are formed at about 3,500 K and have been collected by rockets and Earth satellites7. The diffuse blue rings surrounding sunspots8 are visible evidence of the formation of large numbers of small particles. They must also form in intergranular regions at the base of the photosphere and then be ejected into the upper regions by the violent winds and powerful thermals present.

Smaller particles are segregated from larger because forces

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acting on the projected area have greater effect on lighter particles. These generally have a preponderance of negative charge and therefore produce a surplus of negative electricity at high levels in the atmosphere, corresponding to a surplus of positive charge below9. The interactions of ions and free electrons with these particles is complicated, but it is likely that charges accumulate in the highly conducting atmosphere because the positive particles will rapidly capture free electrons. There will then be a surplus of positive ions which are far more massive than electrons and take much longer to neutralise the equivalent negative charge on the smaller particles, enabling the surplus negative charge to accumulate in regions remote from the positive charge zone. Hydrogen and other elements at this location and temperature are far from fully ionised10 and the increasing voltage gradient due to the charges will eventually cause further ionisation, leading to electrical breakdown. The rate of charging will reduce as the voltage gradient increases, but the forces unleashed by solar activity are so immense that ample energy is evidently available for discharge conditions to be attained.

At any given time there are several million electrical discharges in the photospheric region, each about 1,000-2,000 km long and lasting 10 min, causing the average temperature of the solar 'surface' to rise to 6,000 K. The flow of positive ions in the electromagnetically compressed discharge channels persists for a time after the charge is consumed and the hot gas ascends and extends above the extinct arcs, forming the white patches of granulation. Most of the particles are recycled, as in terrestrial meteorology, but some are ejected to greater heights, aided by radiation pressure, carrying charge with them and building up electrical energy in the corona. The resulting discharges are generally more sporadic and violent, such as long prominences, and these are observed to have the same velocity of propagation as terrestrial lightning, attracting other prominences like currents in parallel conductors. The electrical and magnetic influence of these powerful discharges destroy some areas of the photospheric discharges, forming sunspots, in which the magnetic field pattern is the resultant of the surrounding photospheric discharges and that of the flare above. It is unnecessary therefore to assume that magnetic fields are produced by some unknown process inside the Sun, especially as the generally held view, that the magnetic field is always vertical in the umbra of sunspots, has been disproved by observations11.

The discharges in the perimeter of sunspots show as white streaks bent back by electromagnetic attraction towards the surrounding discharges. Jets from these discharges produce the outward flow of gas known as the Evershed effect, and their maximum observed velocity of 8 km s<sup>-1</sup> agrees with Bruce's calculated value<sup>2</sup>. The photograph of a sunspot (Fig. 1) taken

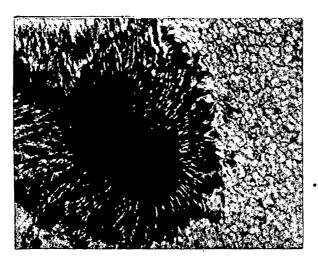


Fig. 1 Sunspot and granulation. (Courtesy Project Stratoscope of Princeton University.)

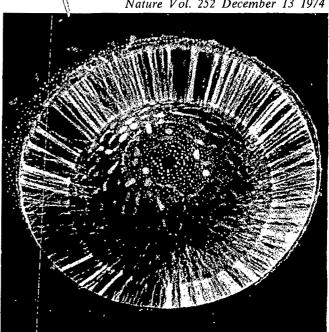


Fig. 2 Annular electrical discharges in a boiler igniter. (Courtesy Colt International Ltd, New Lane, Havant, Hampshire.)

by the Project Stratoscope<sup>12</sup> balloon-mounted telescope clearly shows the white tracks of electrical discharges of a width less than 300 km, the limit of the resolution of the telescope<sup>13</sup>. It is of interest to compare this with the photograph of an annular electrical discharge in a boiler igniter (Fig. 2), which shows several remarkable similarities, considering the enormous difference of scale. Many peripheral sunspot streaks resemble the jets produced by a laboratory plasma projector.

Bruce's theory explains many other solar characteristics in terms of electrical discharges and predicted temperatures of over 108 K in the corona a year before this was confirmed by observations14. He describes many cases of prolonged stellar atmospheric discharges, where the close correlation with observational evidence and similarity to lightning characteristics are most striking. It is an important confirmation of his theory that many planetary nebulae as well as galaxies have straight or spiral arms, evidently formed by discharges in which electromagnetic forces concentrate the matter in their channels. In planetary nebulae this material condenses to form binary partners and planets, while in the vast galactic discharge channels, stars and smaller nebulae are formed. The well known 'ring' type planetary nebula is only one of many different kinds, and if they all evolve in a similar way, then its shape is not that of an expanding shell of gas, but merely the result of a discharge jet happening to be in line, or nearly in line, with the observer. Doppler spectra characteristics show diametrically opposite jets in some of these nebulae.

The theory that solar disturbances are caused mainly by the movements of charged atmospheric particles may explain why the level of activity in the Sun is so closely related to the tidal effects of the planets<sup>15</sup>. Small prominences and certain other solar features seem unlike any discharge process and may be caused by the varying magnetic fields of the large discharges acting on ions and charged particles, particularly when these are near the magnetic focus of deflected current channels.

#### Galactic evolution

If charged particles are ejected or repelled by the hot nucleus of a galaxy for hundreds of millions of years (Hubble stages E0 to E7), a very extended charged atmosphere will develop with an immense store of electrical energy. The galaxy will then be poised on the brink of an enormous atmospheric discharge, in which a small increase in the voltage gradient would start a cataclysmic outburst near the nucleus and the jet would then advance far into the charged atmosphere. The initial main breakdown is likely to be initiated by the gravitational, electrical

or magnetic influence of an active neighbouring galaxy, possibly explaining why some discharge jets appear to point in that direction16. This 'quasar' phase occurs at Hubble stage S0, the point of departure for three types of evolution (Fig. 3). The discharge would very quickly attain peak output lasting for a few million years, during which time a second diametrically opposite discharge would occur, set off either by the extragalactic cause of the original discharge, or as a result of shock waves from the first jet orbiting the nucleus and focusing at the antipodes. Both discharges then continue to extend far into the surrounding negatively charged atmosphere at an average velocity of about 4,000 km s<sup>-1</sup> (ref. 17).

Such a jet, with its myriads of discharge channels (similar to the few that are plainly visible in the great solar flare of June 4, 1946) would engulf a star like the Sun in minutes, heating the entire mass to thermonuclear temperatures and causing the short time variation of energy output which is observed in quasars. Magnetic pinch effects would also produce variations, but over far longer periods, as is evident from the jet of M87. An indication of diametrically opposite jets may be the difference in the values of the red shift of the nucleus, if detectable, which is the cosmological value, and the emission and absorption spectra of the jets, where there is a high relative velocity in line

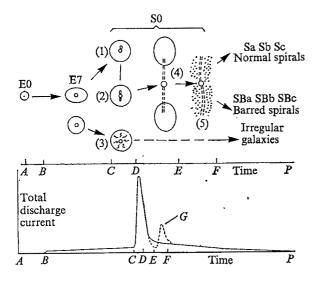


Fig. 3 Galactic evolution by electrical discharge processes. A-C, Long period of slow galactic growth  $(10^9-10^{10} \text{ yr})$  and increasing temperature of its nucleus, which repels charged particles and starts the gradual accumulation of electrical energy. B-C, Atmospheric voltage gradient produces small aggregate leakage current (vertical scale exaggerated) which is limited by the availability of free ions in a very rarified atmosphere. When the maximum value is attained at any particular time and place, the voltage gradient can increase without a corresponding appreciable increase in the leakage current (Townsend effect). The gradient is highest near the nucleus and falls approximately as the inverse cube of the distance (for a distributed charge). C-D, Eventually the increase in the electrical field causes electrons to attain the energy required to produce further ionisation, and catastrophic discharge occurs. This is the quasar phase (1), lasting 106-107 yr. During this time opposite jets form (2). In about 3% of galaxies irregular discharges occur (3). D-E, The extending discharges energise surrounding free electrons to relativistic velocities, which produce radio emission from extensive lobes (4). This is the radio-galaxy phase. E-F, The electrical charge rapidly diminishes and the lobes of radio emission contract. The regions surrounding the discharges cool and clouds of obscuring matter are produced. These absorb the energy of the high temperature core of the discharges and re-emit it as infrared radiation. This is the Seyfert phase (5). F-P, Charge continues to be collected from the galactic atmosphere, but the electro-magnetically compressed discharge channels gradually cool and the matter condenses into the stars and planetary nebulae of the galactic arms. Some galaxies are distorted by impinging extragalactic fields. G, The main discharge may be restricted by the pinch effect, followed by another burst of energy at a lower level. Periodic outbursts (not illustrated) may be caused by repeated charge-discharge processes, helped by the huge forces of the active phase.

with the observer. The magnetic fields of the discharges extend to other galaxies, which frequently show the peculiar shapes caused by the impact of these powerful forces. Perhaps the reversals of terrestrial magnetism and other disturbances in our galaxy are the result of giant discharges in neighbouring galaxies.

The channel of a discharge would acquire a powerful positive charge, causing free electrons in the surrounding atmosphere to accelerate towards it, attaining relativistic velocities in extensive lobes on each side of the nucleus, as the quasar evolves into a radio galaxy (after Ryle<sup>18</sup>). Synchrotron emission by electrons in the extensive magnetic fields of the discharges would contribute to the total radiation. The flow of current following the characteristically 'brief' peak period of a few million years would be maintained by the collapsing magnetic field and the slower lateral current infeed from the surrounding charged zones. In straight discharges, matter from the jets then accumulates near the ends of the arms, as seen in NGC2859, but in discharges which are deflected by the field of adjacent galaxies or by rotation, the ejected matter continues in orbit about the nucleus, forming spiral arms. The intermediate Seyfert stage is illustrated in Fig. 3. A few cases are known where the spiral structure has apparently been formed by tidal forces. If random major discharges occur in the absence of a single main initial discharge due to an external influence or rotation, an irregular galaxy is formed.

The energy produced by the discharges is largely from thermonuclear reactions caused by high temperatures and electromagnetically increased pressures. No other known processes in astronomy can account adequately for the extent of nuclear synthesis required19. The energy was estimated by Bruce as 1060 to 1061 erg (ref. 2) on the basis of mass loss, which compared well with later estimates by others involving radiation measurements20. As in the stellar cases, many theoretical deductions by Bruce were confirmed by observations2. He has gone on to conjecture that strings of galaxies were formed as condensations in the universal discharge channels of the original Big Flash, and to predict that these galaxies have a higher content of heavy elements after their long subjection to thermonuclear reactions, compared with galaxies formed more slowly by gravitational aggregation outside the discharge channels.

### Validity of theory

Although in recent years many astronomers have suggested the time is ripe for a revolutionary new approach to clear up the many problems in astrophysics, few have commented on Bruce's work, presumably because it cannot be properly understood without some knowledge of the characteristics of lightning and other electrical discharges. Bruce was in the unusual and fortunate position of having studied these subjects intensively, his publications earning him a DSc. He then concentrated on the applications to astronomy, and was able to do this without preconceived ideas. His position outside the accepted astronomical organisations has, however, made it more difficult for his views to become widely known. One objection is that his theory is not supported by any detailed mathematical studies, but until it is better known and understood, these are unlikely to be attempted.

Perhaps this article will encourage workers in many fields of astrophysics to take a closer look at Bruce's work and to develop it in greater detail. If they will start by assuming it is possible for electrical charging and discharging to take place, then see where this will lead, perhaps the validity of their assumption would soon be established and C. E. R. Bruce would obtain the recognition he deserves.

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# articles

### The coenzyme-binding domains of glutamate dehydrogenases

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A combination of secondary structure predictions and sequence comparisons with dehydrogenases of known structure allows two coenzyme-binding domains to be located in each of the sequences of bovine and Neurospora glutamate dehydrogenases. One domain shows significant sequence homology with the structurally-similar domain of glyceraldehyde-3-phosphate dehydrogenase.

Amino acid sequences of three glutamate dehydrogenases (GDHs) have been determined: the bovine liver (500 residues) and chicken liver<sup>2</sup> (503 residues) enzymes, and recently the NADP-dependent enzyme from the fungus Neurospora crassa³ (452 residues). All three are hexamers of identical subunits. Bovine and chicken GDHs are strongly homologous, differing only in 30 residues<sup>2</sup>, and although bovine GDH will be considered here the conclusions apply equally to the chicken enzyme. The Neurospora enzyme shows clear but limited homology with the vertebrate GDHs in the N-terminal two-thirds of the chain<sup>3,4</sup>, suggesting that the fungal and vertebrate enzymes shared a common evolutionary origin. The Neurospora GDH differs from the vertebrate enzymes in allosteric properties and in being NADP dependent. Vertebrate GDHs can utilise either NAD or NADP. Like many other microorganisms, Neurospora has a distinct NAD-dependent GDH which will not be considered here.

Knowledge of the three-dimensional structure of these enzymes is desirable but a crystallographic structure determination of any GDH is very remote. Large crystals and preliminary diffraction data have been obtained for the Neurospora enzyme (I. D. A. Swan, A. C. T. North and J. C. W., unpublished) but the crystal form examined had a large asymmetric unit and complex unit cell. As an interim measure I report here predictions of secondary structure in GDHs and sequence comparisons with other dehydrogenases of known structure. Taken together these two approaches allow the clear identification in each of the bovine and Neurospora GDH sequences of two structural domains (termed domain-1 and domain-3 because of their

positions in the sequence) which resemble the coenzyme-binding domains of other dehydrogenases.

The NAD-binding domains are of very similar structure in lactate dehydrogenase<sup>5</sup> (LDH), soluble malate dehydrogenase<sup>6</sup>, liver alcohol dehydrogenase7 (ADH) and muscle glyceraldehyde-3-phosphate dehydrogenase8 (GPD) and an analogous domain binds ATP in phosphoglycerate kinase9,10. A description and schematic diagram of this common domain is given by Rossman et al.11. In different enzymes it varies between about 110 and 160 residues and contains a central six-stranded parallel β sheet. The  $\beta$  strands in their order in the sequence are termed  $\beta A$ ,  $\beta B$ ,  $\beta$ C,  $\beta$ D,  $\beta$ E and  $\beta$ F. The strand order in the  $\beta$  sheet in the above enzymes is CBADEF. The β strands are interconnected by more variable loops containing α helices and in some cases β structure (Fig. 1). The ABC half of the domain is related to the DEF half by an approximate twofold axis, and each half has been termed a mononucleotide binding unit11. Domains with central parallel  $\beta$  sheet, some of which also have a nucleotide-binding function, have been described in other proteins11, but these differ from the dehydrogenase domains in the number or order of the  $\beta$  strands. Rossman et al.11 propose that all these domains share a remote common ancestor in precellular evolution.

Secondary structure predictions

Of several available methods<sup>12-16</sup> for predicting the positions in protein sequence of  $\alpha$  helices and  $\beta$  structure, I have used the method of Chou and Fasman<sup>12,13</sup> which derives separate rules for the nucleation and stability of  $\alpha$  and  $\beta$  structures from the frequency of occurrence of residues in these structures in 17 proteins. In a comparison of several prediction methods applied to the parallel β sheet domain of adenylate kinase<sup>17</sup>, this method was as successful as any and compared well with the pooled results of the different methods. I have tested the applicability of the method to parallel \beta sheet domains by making predictions for the domains of known structure in ADH, GPD and LDH (Fig. 1), and by comparison of the number of residues found in the parallel \beta strands of six proteins with the numbers expected from the  $\beta$  potentials of Chou and Fasman<sup>12,13</sup> (Table 1). To simplify my report, predictions of bends will be omitted because they do not significantly affect the proposals for the GDHs.

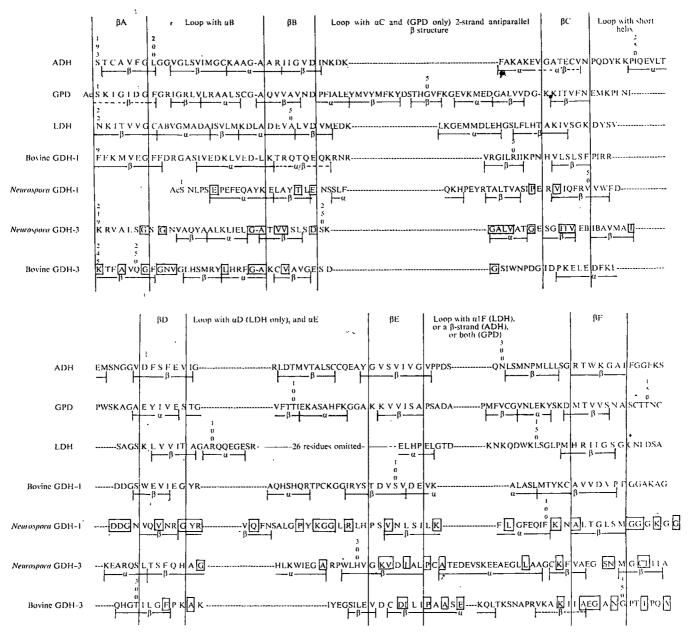


Fig. 1 Alignment of coenzyme-binding domains. The sequences (in one-letter code<sup>18</sup>) are of horse liver ADH (ref. 27), lobster muscle GPD (ref. 28), dog-fish M<sub>4</sub> LDH (ref. 19), bovine liver GDH (ref. 1) and Neurospora NADP-dependent GDH (ref. 3). In the LDH sequence, 26 residues (not included in ref. 19) are omitted because different published versions conflict<sup>11,15</sup>. Except for some loop sequences the alignments of ADH, GPD and LDH are taken from ref. 11. Predictions of  $\alpha$ -helix and  $\beta$ -structure are indicated beneath each sequence. Dashed lines indicate ambiguities or tentative predictions (see text). Boxed residues emphasise the following sequence identities: in Neurospora GDH domain-1 with bovine GDH domain-1; in Neurospora GDH domain-3 with GPD; in bovine GDH domain-3 with Neurospora GDH domain-3. Precise residue-by-residue alignment can be deduced by counting residues from the nearest vertical line.
\* Reactive residues.

The overall frequencies of residues in parallel β sheets (Table 1) agree very well with expectation, the only significant deviations being an excess of asparagine (and perhaps also of valine and isoleucine) and deficiencies of glutamine and methionine. The distribution of residues along parallel β strands in these six proteins shows tendencies for lysine to favour the N-terminal ends and for glutamate and aspartate to favour the C-terminal ends. If the published  $\beta$  potentials<sup>12,13</sup> for lysine, glutamine, asparagine, glutamate and aspartate are modified to accommodate these discrepancies and tendencies, however, no β-prediction for the domains of ADH, GPD and LDH is altered by more than two residues. Therefore I have used the published β potentials without modification, taking—where given—the values based on 17 proteins13 rather than those based on 15 (ref. 12).

I have also tried a less demanding nucleation condition to take into account the excess of valine and isoleucine: that any two of valine and isoleucine out of three residues is a possible

 $\beta$  nucleus. This condition permits  $\beta$  predictions approximately corresponding to BA of GPD and to the putative BF of bovine GDH domain-3, which are not otherwise predicted as  $\beta$  strands, but this condition should not be applied generally without further tests. Predictions using this rule, and two ambiguous predictions, are indicated by dashed lines (in Fig. 1). The ambiguities arise in cases where two almost completelyoverlapping sets of residues have high and almost equal average α and β potentials. In other cases where predictions of high  $\alpha$  and  $\beta$  potential overlap, the residues have been resolved into an a section (minimum lengths six residues) and an adjacent β section (minimum length five residues) provided these sections are individually consistent with the rules of Chou and Fasman<sup>13</sup>. The rules are somewhat ambiguous in defining the ends of a and β sections and in some predictions in Fig. 1 up to two end residues could be added or subtracted arbitrarily.

The assignments for ADH, GPD and LDH (Fig. 1) show that the method is largely successful in predicting the approximate,

Table 1 Comparison of the numbers of residues observed in the parallel  $\beta$  strands of six proteins with the numbers expected from the  $\beta$  potentials of Chou and Fasman<sup>12,13</sup>

	Summed composition	Number of residues found	Expected
	of the six proteins	in parallel β sheet	number
Ala	140	19	18.8
Arg	41 •	4	5.1
Asn	51	10	5.3
Asp	84	9	9.3
Cys	27	4	4.4
Gln	46	i	7.0
Glu	88	$\dot{7}$	4.0
Gly	157	19	17.3
His	27	3	2.6
Ile	88	25	
			19.5
Leu	114	13	19.2
Lys	124	17	12.7
Met	44	5 7	10.2
Phe	50	7	10.6
Pro	67	1	4.2
Ser	132	16	14.6
Thr	85	11	14.1
Trp	13	2	2.1
Tyr	37	$\tilde{\epsilon}$	6.6
Val	169	47 .	38.5
Total	1,584	226	226.1

The proteins, and the references to the structural information, are horse liver ADH<sup>7,27</sup>, lobster muscle GPD<sup>8,28</sup>, dogfish M<sub>4</sub> LDH<sup>5,19</sup>, porcine adenylate kinase<sup>21</sup>, *Peptostreptococcus elsdenii* flavodoxin<sup>32</sup> (aligned by homology with the *Clostridium* MP protein alignment<sup>11</sup>), and subtilisin Novo<sup>32</sup>. The expected numbers are calculated as described by Chou and Fasman<sup>12</sup>, using the summed amino-acid compositions of the six proteins (first column).

positions of  $\alpha$  and  $\beta$  sections, although the ends of the predicted sections are usually inaccurate by a few residues. Of the 18 ß strands in the parallel sheets the approximate positions for 14 (or 15 if the specific nucleation condition is used) are successfully predicted. Of the remaining three  $\beta$  strands,  $\beta$ C of ADH has an ambiguous rather than an incorrect assignment, and βD of GPD, although predicted as α helix in the lobster sequence (Fig. 1) is predicted as  $\beta$  in the homologous pig and yeast enzymes. In the loops between the  $\beta$  strands many predictions are approximately correct, but in several cases part or all of some  $\alpha$  helices, particularly  $\alpha B$  and  $\alpha E$ , are predicted as  $\beta$ . The consistent occurrence of this type of incorrect prediction is interesting because it suggests that the stability of some of the a helices may depend on long range constraints involving parts of the domain which are remote in the sequence. The general success of this and other prediction methods<sup>13,15</sup> is dependent on most secondary structure in the proteins being determined by short range interactions.

#### Alignment of the GDH domains

Application of secondary structure predictions to the bovine and Neurospora GDH sequences revealed two candidates in each sequence for parallel  $\beta$  sheet domains. These resemble the known coenzyme-binding domains in being 100-150 residues long, very rich in secondary structure with a tendency for  $\alpha$  and  $\beta$  sections to alternate, in having candidates for bends only near the ends of  $\alpha$  and  $\beta$  sections and in the absence of long sections predicted as random coil. Secondary structure patterns of this type are not in themselves definitive of parallel β sheet domains because they could perhaps arise by chance or from other types of structure. Confidence in the conclusion that these parts of GDH are coenzyme-binding domains comes from the occurrence at approximately the correct positions of critical residues which are functionally important in coenzyme binding in other dehydrogenases11, and in the case of domain-3 from sequence homologies with the GPD domain.

The critical residues<sup>11</sup> used as criteria of alignment of the GDH domains are (Fig. 1, numbered for ADH), glycine 199, glycine 204 (conservatively replaced by alanine in *Neurospora* GDH domain-3), aspartate 223 (conservatively replaced by

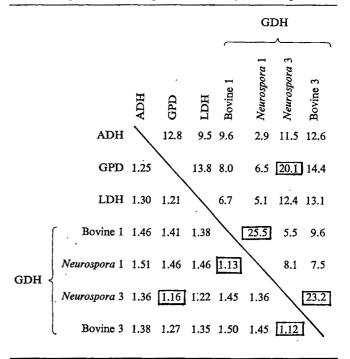
glutamate in three of the GDH domains), and one or other of glycine 261 or glycine 270. Also used are the hydrophobic residues which occur in most but not all cases at the third and fifth positions from the N-terminus of each of the parallel  $\beta$  strands<sup>11</sup>.

Approximate alignments of the GDH domains were initially based on tentative identifications of the six parallel  $\beta$  strands from the secondary structure predictions, using the following criteria of the lengths of loops between the parallel  $\beta$  strands. First, the  $\alpha B$  loop is always 17 or 18 residues; second, the  $\beta C$  to  $\beta D$  loop is not shorter than 8 residues, as in LDH; third, no other loop is shorter than 10 residues. Twelve residues in the  $\alpha C$  loop of ADH is the shortest other loop of known structure, but ten-residue loops are structurally plausible.

Surprisingly, in the case of bovine GDH domain-1 only one approximate alignment was consistent with both the secondary structure predictions and the loop criteria, and this was slightly refined to align critical and hydrophobic residues, giving the alignment shown in Fig. 1. In this domain the  $\beta$ C,  $\beta$ E and  $\beta$ F regions may be inaccurate by a few residues, but no grossly-different alternatives satisfy the criteria. Minor adjustments could slightly increase the sequence similarities to ADH or GPD but at present these are not justified. *Neurospora* GDH domain-1 was aligned by sequence homology with bovine GDH domain-1. It lacks a candidate for the  $\beta$ A region but otherwise satisfies all the criteria well (Fig. 1).

In the case of domain-3 of both GDHs, several alternative approximate alignments, differing in the lengths of the loops, were equally consistent with the secondary structure predictions and loop criteria. Fortunately one of these alignments of *Neurospora* GDH showed sequence homology with the domains of the three GPD sequences, and the same alignment also conserved the critical and hydrophobic residues. These criteria give considerable confidence that this alignment of *Neurospora* GDH domain-3 (Fig. 1) is essentially correct, the only uncertainty

Table 2 Sequence relationships between coenzyme-binding domains



Pairwise comparisons of the alignments in Fig. 1 are expressed as (upper right of matrix) number of identical residues per 100 residues compared, and (lower left of matrix) minimum base changes per codon compared. Numbers for GPD are the average of the three comparisons with the lobster muscle<sup>28</sup>, pig muscle<sup>29</sup> and yeast<sup>30</sup> sequences, the latter two being unambiguously aligned by homology with the lobster GPD alignment in Fig. 1. Boxes enclose the comparisons showing strongly significant relatedness.

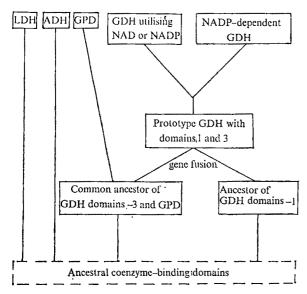


Fig. 2 A model of the early evolution of glutamate dehydrogenases.

being the exact location of the  $\beta D$  region where the sequence homology is weakest. Bovine GDH domain-3 was aligned by sequence homology with this *Neurospora* domain, and its alignment (Fig. 1) also conserves the critical and hydrophobic residues. The sequence identities in these alignments are emphasised by boxes in Fig. 1.

Part of bovine GDH domain-3 ( $\beta$ A to  $\beta$ B) was identified and aligned by Rossman *et al.*<sup>11</sup> on the basis of the sequence homology with GPD in this region, which includes the conservation of critical residues. In the bovine case this homology does not extend markedly to the rest of the domain. My arguments demonstrate that conclusions based on weak sequence homology can be considerably strengthened by secondary structure predictions which may in favourable cases provide a rational basis for the assignment of gaps to appropriate parts (such as loops) of sequence alignments.

#### **GDH** domains and enzymology

Direct coenzyme-binding measurements<sup>20,21</sup> suggest that bovine GDH has two coenzyme-binding sites per polypeptide chain, one involved in enzyme activity with a high affinity for both NADH and NADPH, and the other of low affinity for NADH and very low affinity for NADPH. Similar measurements on Neurospora GDH (ref. 22 and B. Ashby, J. C. W., and J. R. S. Fincham, unpublished) have shown a stoichiometry of one molecule of NADPH bound per polypeptide chain in the conditions tried, but the inactivation of this enzyme by NADPH<sup>23</sup> is perhaps consistent with the presence of a second NADPH-binding site.

The alignment of domain-1 of the GDHs unexpectedly located the reactive lysine (126 in bovine, 113 in Neurospora) in the bend sequence immediately following BF (Fig. 1). In both enzymes<sup>24-26</sup> this lysine has an abnormally low pK and is highly reactive to pyridoxal-5'-phosphate. Modification of the lysine inactivates the enzyme and bound coenzyme protects against this inactivation. The reactive cysteines of LDH and GPD are in closely equivalent locations (Fig. 1), which places the reactive group close to the nicotinamide end of the bound coenzyme. In the equivalent bend of ADH lysine 323 occurs in a sequence similar to that of lysine 126 of bovine GDH (-Phe-Gly-Gly-X-Lys-). ADH lysine 323 is not known to be reactive. The reactive lysines of GDH could have either a catalytic role (as suggested for cysteine 148 of GPD (ref. 8)) or a location in which modification sterically interferes with coenzyme binding (as suggested for cysteine 164 of LDH5).

If GDH is analogous to LDH and GPD in the structural

relationship of the reactive residue to coenzyme binding, it follows that domain-1 is the site involved in activity. The alternative possibility cannot be excluded: that domain-3 is the site involved in activity and that the reactive lysine projects into domain-3 so that its modification interferes with coenzyme binding.

Although *Neurospora* GDH domain-1 lacks a sequence for  $\beta A$  and is most simply interpreted as a five-stranded  $\beta$  sheet domain, it is a good candidate for an NADP-specific binding site. Current evidence suggests that in this *Neurospora* GDH, the primary interaction in coenzyme-binding is with the 2'-phosphate of the adenosine ribose. NADH binding is not detectable (ref. 22 and B. Ashby, unpublished). 2'-AMP, but not 5'-AMP, is an inhibitor showing competitive kinetics with NADP (ref. 22), but the relatively low affinity for 2'-AMP suggests that interactions at the nicotinamide end of the coenzyme are also involved. It is possible that  $\beta A$ , which interacts with the adenosine ribose in NAD-binding domains, is dispensable in NADP-specific domains.

The putative domain-2, consisting of the sequence between domain-1 and domain-3, is the candidate for the functions of catalysis and 2-oxoglutarate or L-glutamate binding. It shows a slightly greater degree of sequence homology between bovine and *Neurospora* GDHs (ref. 3) than do domains 1 and 3. The possible domain-4, comprising the sequence from domain-3 to the C-terminus of the protein, shows no sequence homology and is completely different in secondary structure predictions in the bovine and *Neurospora* enzymes.

### **Evolutionary implications**

Table 2 shows the sequence relationships between the coenzyme binding domains aligned as in Fig. 1, expressed both as percentage of identical residues and as minimum base changes per codon. On either criterion three comparisons show a strongly significant genetic relatedness which provides evidence for a common evolutionary origin of the two sequences compared: bovine GDH domain-1 with Neurospora GDH domain-1, bovine GDH domain-3 with Neurospora GDH domain-3, and Neurospora GDH domain-3 with the domains of the three GPD sequences. Remarkably, Neurospora GDH domain-3 is almost as closely related to the GPD domains as it is to bovine GDH domain-3. The relatedness of bovine GDH domain-3 to the GPD domains appears much less strong because the homology is mainly restricted to the  $\beta A$  to  $\beta B$  region of the domain. The homologies between GDH and GPD suggest that the lines leading to the vertebrate and the NADP-dependent fungal GDHs diverged from each other very early in evolution, possibly at a precellular stage. A model for the precellular evolution of GDH is proposed (Fig. 2) in which domain-1 and domain-3 occurred initially on separate evolutionary lines and later joined by gene fusion at or after the time of divergence of GDH domain-3 from GPD.

Weakly significant relatedness also occurs between Neurospora GDH domain-3 and LDH, ADH and GPD, and LDH and GPD. This probably reflects a remote precellular common ancestry of all these domains<sup>11</sup>. Caution is required in the interpretation of weak sequence similarities because it is not known to what extent strong structural constraints on sets of residues can result in convergent evolution in different lines. Bovine and Neurospora GDHs domain-1, although strongly related to each other, are not significantly related to any of the other domains. This may reflect detailed faults in the alignments of Fig. 1, if these are out of phase in places with the true evolutionary alignments. Alternatively GDH domain-1 may have accepted mutations at a faster rate than other domains during the evolution of the utilisation of NADP.

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### Comparison of classical and autogenous systems of regulation in inducible operons

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The mechanism of autogenous regulation, whereby a protein directly controls the expression of its own structural gene, is compared on the basis of function with the classical mechanism, in which the structural gene of the regulator is itself unregulated. The autogenous mechanism is superior to the classical one in inducible catabolic systems governed by a repressor; the opposite is true if the regulator is an activator.

CONTROL of transcription by a constitutively synthesised repressor protein is central to Jacob and Monod's model for the regulation of gene expression. Inducible operons were the first to be understood at this level of molecular detail in bacteria, and in several of the best studied examples, such as the lactose<sup>2</sup>, galactose<sup>3</sup> and glycerol<sup>4</sup> operons, the classical Jacob-Monod mechanism is believed to be operative. Alternatives, however, have been discovered: the regulator protein in some cases—such as the arabinose5, maltose6 and Dserine deaminase7 operons—is an activator, or positive element, in the control system. In other cases, for example, the histidine utilisation system<sup>8</sup>, there is a repressor mechanism but the regulator protein is directly involved in modulating the expression of its own structural gene. This latter feature of the hut system provides an example of a more general class of phenomena that has been called autogenous regulation9.

What are the functional implications of these differences? The relative merits of genetic control by repressors and activators have been explored elsewhere<sup>10</sup>. Here I shall examine the functional implications of control by a constitutively synthesised regulator and by an autogenously regulated one. To answer questions of this type one cannot compare directly two representative systems (for example, lac and hut) because they have numerous differences that are irrelevant to the comparison of classical

and autogenous regulation per se. A controlled comparison in which the two systems are identical in every respect except for the difference in regulatory mechanism is desirable but difficult to achieve experimentally. Nevertheless, such comparisons can be simulated by mathematical analysis. I have performed such an analysis comparing models of classical and autogenous regulation in inducible operons11. Before discussing the results I shall point out the criteria for functional effectiveness that I have used in comparing these systems.

### Criteria for functional effectiveness

To be specific, I will consider inducible catabolic systems in bacteria that allow the cell to utilise nutrients found in its environment. Several criteria can be formulated for functional effectiveness. (1) A sharp threshold in the concentration of the substrate necessary for induction protects the organism from wasteful synthesis of the catabolic enzymes when the substrate level is so low that insufficient benefit would be gained from induction. (2) The ability to make the most product available to the organism from a given supra-threshold increment in substrate is clearly advantageous to the organism whenever that substrate is the only nutrient available for growth. (3) Stability is obviously essential for a system to function properly. (4) A temporally responsive system can be induced rapidly, which is an advantage when substrate levels change abruptly. (5) Insensitivity to perturbations in the system's component parts ensures that the system will continue to function in spite of the continual perturbations it experiences in any real environment. These perturbations result from non-lethal mutations as well as physical changes in temperature and so on. None of these criteria assumes anything about the type of regulatory mechanism involved.

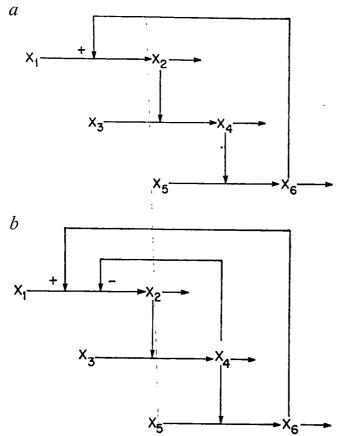


Fig. 1 Schematic models of inducible catabolic systems. a, Classically regulated operon in which the structural gene of the regulator protein lies outside the operon and is regulated independently of the operon. b, Autogenously regulated operon in which the structural gene of the regulator is located within the transcriptional unit that the regulator controls.  $X_1$ , Nucleotide precursors;  $X_2$ , specific mRNA;  $X_3$ , amino acid precursors;  $X_4$ , enzymes of the catabolic pathway (plus regulator in the case of autogenous regulation);  $X_5$ , substrate;  $X_6$ , product-inducer. Although it is not critical for the comparisons reported here, the product has been chosen as inducer because in most cases the inducer is known to be a metabolic product rather than the substrate of the pathway<sup>12</sup>. The horizontal arrows indicate chemical transformations at the mRNA, enzyme and metabolite levels. The vertical arrows represent modifier or catalytic influences (the sense of the influence is positive unless otherwise indicated). Except for the differences in regulation these models are assumed to be identical.

### Comparison of repressor-controlled systems

First, I will compare inducible systems involving repressors that are regulated in the classical manner with those that are regulated autogenously. The two possibilities are represented schematically in Fig. 1. Except for the differences in regulation these models are assumed to be identical.

These two models can be compared under various conditions, and because there are several criteria to consider, a meaningful tabulation of all the results can present a problem. Figure 2, however, is a simple diagram summarising many of the results of these comparisons. This is a two-dimensional plot with the vertical axis representing the strength of the inducer's contribution to the control of transcription and the horizontal axis representing the strength of the autogenous contribution. Each point in this space can be thought of as representing a different system with distinct properties. This space, however, can be divided into subclasses of systems that have similar properties. Since I am comparing inducible systems that utilise a repressor mechanism, the inducer has a positive effect on transcription (g<sub>2d</sub>>0) while the autogenous effect is negative (g<sub>24</sub><0).

Thus, I can restrict my attention to the upper left-hand quadrant of this figure. The vertical axis in this quadrant is the locus of points representing classical systems, since the autogenous contribution is zero. Line (a) represents another classification. The position of this line is determined by analysing the stability of these models. All systems represented by points below line (a) are stable (if perturbed momentarily they will return to their predisturbance condition) whereas all those above this line are unstable (they will not return to their predisturbance condition).

Lines, such as (b), radiating from the point where line (a) intersects the horizontal axis, are lines of equivalence with respect to the first two criteria. All systems whose regulatory parameters have values that determine points on a given line, such as (b), have in common: (1) the same sharpness of threshold in substrate concentration for induction and (2) the same product formation for a given increment in substrate. The slope of the line is a quantitative measure of these properties. The steeper the slope is, the sharper will be the threshold and the greater the product formation.

Systems of the two types now can be compared. As noted before, all of the systems represented on a given line such as (b) behave identically according to the first two criteria. They differ, however, according to the third criterion. The position of the classical system is closer to the boundary of instability than are those of the corresponding autogenous systems represented in Fig. 2. In other words, line (b) and the boundary of instability, line (a), diverge to the left and the autogenous systems lying further from this boundary are more stable.

The fourth criterion, that of temporal responsiveness, is examined in Fig. 3. Curve (a) represents the response of the classical system. The system is in a steady state before t=0. At time zero the substrate concentration is suddenly increased to a new value that is maintained throughout the experiment. The concentration of the product is plotted as a function of time. Similar responses are plotted for a series

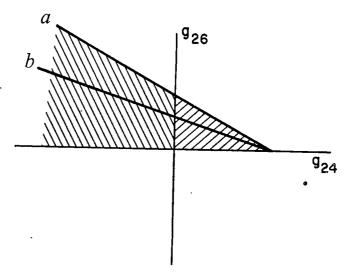


Fig. 2 Graphical comparison of systems with alternative types of regulation. The vertical axis represents the strength of the product's contribution to the regulation of transcription and the horizontal axis represents the strength of the autogenous contribution to this regulation. Line (a) represents the boundary of instability. Line (b) is a representative line of equivalence with respect to the first two criteria for functional effectiveness. The vertical axis is the locus of points representing the classically regulated systems with either activator or repressor control. The shaded area to the left of this axis is the location of points representing stable systems with autogenously regulated repressors. Similarly, the shaded area to the right of the vertical axis represents stable systems with autogenously regulated activators. See text for further discussion.

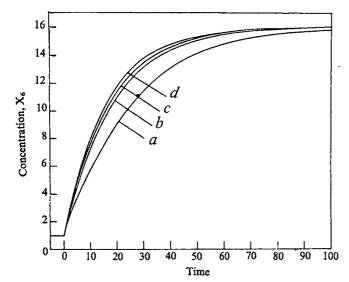


Fig. 3 Temporal responsiveness of equivalent systems with different strengths of autogenous regulation of repressor. The strength is represented by the parameter  $g_{24}$ , which has values: a, 0.0; b, -0.5; c, -1.0; d, -2.0. The corresponding values for the parameter  $g_{26}$  are chosen so that the points representing the different systems all lie on the same line of equivalence. Curve (a) represents the classical system with constitutively synthesised repressor. See text for further discussion.

of systems with increasing strengths of autogenous regulation and represented by points on the same line of equivalence (see Fig. 2). Systems with a high degree of stability tend to have more sluggish temporal responses to change. Figure 3 shows that the opposite relationship is true of systems with autogenous regulation of repressor.

Finally, a comparison on the basis of the fifth criterion shows that the sensitivities of the system with autogenous regulation of repressor to various perturbations in the structure of the system itself are always less than or equal to the coresponding sensitivities of the classical system.

These comparisons show that the autogenously regulated systems are equivalent or superior to the corresponding classical system according to all five criteria for functional effectiveness of an inducible catabolic pathway.

### Comparison of activator-controlled systems

Quite different results are obtained when classical and autogenous regulation are compared in systems governed by an activator protein. Schematic models representing these two types of systems are identical to those in Fig. 1, except that the negative sign is now positive, to indicate that the regulatory protein X4 has a positive effect on the transcription process. Again, except for the differences in regulation these models are assumed to be identical. The functions of these two models can be compared as in the previous section. Since the autogenous effect on transcription is now positive, I will restrict my attention to the upper right-hand quadrant in Fig. 2. The classical systems are represented along the vertical axis with zero autogenous contribution. Line (a) is the boundary of instability, so the stable systems of physiological interest here are represented by points within the shaded triangular area.

All systems represented on a line of equivalence such as (b) are equivalent with respect to the first two criteria. Since lines of equivalence, such as (b), and the boundary of instability, line (a), converge to the right, systems with increasing strength of autogenous regulation are represented nearer the boundary of instability than the corresponding classical system. Systems with autogenous regulation also

show a more sluggish temporal response to change, as Fig. 4 shows. Curve (a) represents the response of the classical system to an increase in substrate. The temporal responses are slower for the corresponding systems with increasing strengths of autogenous regulation (curves b and c). Finally, a comparison on the basis of the fifth criterion shows that the sensitivities of the system with autogenous regulation of activator are always greater than or equal to those of the corresponding classical system.

Thus, the results in this section are the opposite of those previously obtained for systems governed by a repressor protein. According to all five criteria for functional effectiveness, the classical system is equivalent or superior to the corresponding autogenously regulated systems.

#### **Predictions and observations**

The functional differences I have discussed have obvious implications for the natural selection of classical and autogenous mechanisms of regulation in simple, inducible catabolic systems of the type represented in Fig. 1. Systems governed by an activator protein are not expected to utilise autogenous regulation. Activator-controlled systems without such regulation are superior to autogenously regulated but otherwise equivalent systems by all the criteria given for functional effectiveness. On the other hand, systems governed by a repressor protein can be predicted to involve autogenous regulation as well. Systems with autogenously regulated repressor are superior to classical but otherwise equivalent systems by all of these same criteria. How well do these predictions agree with experimental observations?

The first prediction agrees with what is known about activator-controlled systems in enteric bacteria. There is no known instance of autogenous regulation in such systems. In the best studied cases, the arabinose<sup>3</sup> and maltose<sup>6</sup> operons, the structural gene for the activator is located outside the transcriptional unit(s) known to be under its control. [The regulator protein of the ara operon is both an activator and a repressor. However, the predominant functional properties are those of an activator, and for our purposes the ara operon can be considered a (modified) activator-controlled system.] Thus, a simple, autogenously regulated operon is excluded. One could, of course, postulate more complex models of autogenous regulation in

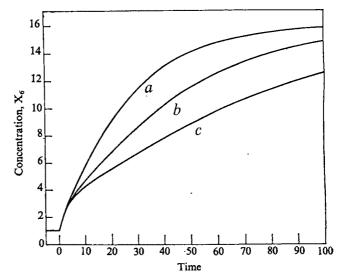


Fig. 4 Temporal responsiveness of equivalent systems with different strengths of autogenous regulation of activator. The strength is represented by the parameter  $g_{24}$ , which has values: a, 0.00; b, 0.15; c, 0.20. The corresponding values for the parameter  $g_{26}$  are chosen so that the points representing the different systems all lie on the same line of equivalence. Curve (a) represents the system with constitutively synthesised activator. See text for further discussion.

which the activator itself is autogenously regulated and its level varies with the expression of the structural genes in another transcriptional unit under its control. Functionally, this type of regulon model would be indistinguishable in most respects from the simple model in Fig. 1b. There is no evidence, however, to support or reject such a model.

The second prediction, concerning repressor-controlled systems, agrees only partially with available evidence. For the histidine utilisation system in Salmonella typhimurium, the evidence clearly agrees with the prediction of autogenous regulation. The structural gene for its repressor is located within one of the transcriptional units controlled by the repressor8. The expression of the second unit also varies with the level of the repressor13, which is functionally equivalent to autogenous regulation for most purposes, as previously indicated. There is no evidence for autogenous regulation in the other two well-studied repressor-controlled systems, the lactose<sup>2</sup> and galactose<sup>3</sup> operons. In each case, the structural gene for the repressor lies outside the only transcriptional unit known to be under its control, so that simple autogenous regulation appears to be excluded. This discrepancy might be explained in one of two ways. The systems might be regulated in a way that is functionally equivalent to autogenous regulation (one possibility has already been mentioned). Alternatively, the systems might have additional, as yet unknown, functions that are not reflected in the criteria for functional effectiveness and that require that the repressor not be autogenously regulated. The first explanation indicates that information about the molecular mechanisms is incomplete, while the second implies a lack of information about the physiological functions of these operons.

With regard to this last possibility, it is already clear that the gal operon is also a component of a much more complex system involved in the biosynthesis of capsular polysaccharide14. Recent evidence indicates that this operon is under the control of two repressors, specified by the galR and capR genes, respectively18. Thus, it would seem that the gal operon cannot be considered functionally as a simple, inducible catabolic system. Similarly, the lac operon

might yet be implicated in additional (dispensible) functions, possibly through the expression of the gene for the transacetylase enzyme, the function of which is presently unknown16.

It has been shown that autogenous regulation has certain advantages and disadvantages and that these have obvious implications for the natural selection of control mechanisms in inducible operons. There is reasonably good agreement of these predictions based on simple models of inducible catabolic systems with current observations of such operons in enteric bacteria. In those cases where the systems appear to be more complex, additional information about molecular mechanisms and physiological functions will be necessary before definitive comparisons can be made.

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### Seismic precursors before rock failures in mines

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Seismic precursors (such as anomalous V<sub>p</sub> values and/or seismic activity) whose behaviour is qualitatively similar to those reported to precede earthquakes are observed before rock failures in dry underground mines. Similar processes may be involved during failure of rock in the mine and the earthquake, and diffusion of fluids into the focal region of a potential earthquake may not be a necessary condition to produce most of the precursors reported.

EVIDENCE is presented here that seismic precursor effects, such as anomalous  $V_p$  values and/or seismic activity, are observed before rock failures in underground mines. Seismic precursor information for rock bursts in a deep (5,000 feet) silver mine in northern Idaho and a roof fall in a Pennsylvania coal mine is discussed. These mines are dry and the effects of fluids on the seismic precursors are precluded.

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### Rock bursts at Galena

The Galena mine is located in the Coeur d'Alene mining district in northern Idaho. Silver, lead and zinc are the predominant metals mined from steeply dipping veins in the Belt formation series of Precambrian quartzites and argillites. A horizontal cutand-fill mining system is used, with the mill tailings being pumped back into the mine to become the floor for the succeeding cut as mining proceeds from a lower level to a level 300 feet above. Detailed information relating to this type of mining system is available elsewhere1.

Rock bursts in the Galena have occurred in dry pillars at depths of 2,000 to 4,900 feet. In this article, rock bursts are qualitatively identified as small bursts (no damage to adjacent mine structures), bursts (no major damage to adjacent mine structures) and major bursts (substantial damage incurred to the adjacent mine structures). Past experience at the Galena has shown that the number of damaging rock bursts increases dramatically once the pillar thickness has been reduced to

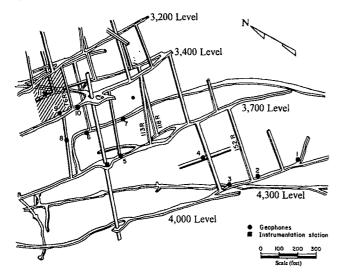


Fig. 1 Geophone locations and mine layout at the Galena mine<sup>2</sup>.

approximately 50 feet. Computer simulation of this mining method has also shown that the maximum principal stress reaches its peak value once the pillar thickness is reduced to 50 feet (ref. 2).

During 1968, detailed microseismic monitoring of potential rock burst zones at the Galena was begun by the United States Bureau of Mines<sup>2</sup>. To monitor a large part of the mine, geophones were placed on three different levels (3,400, 3,700 and 4,000 feet). Figure 1 shows the geophone locations (1-11) and the location of the monitoring station. Some of the results from one active zone (76R) are shown in Figs 2, 3 and 4. In Fig. 2, seismic activity, or equivalently rock noise count against time and pillar thickness, is indicated. The rock noise counts represent small rock failures in the immediate vicinity of the pillar. Note that typically little or no seismic activity is observed during the sandfill operation since the state of stress in the vicinity of the pillar is not changing. Cumulative plots of rock noise source locations are shown in Fig. 3. Seismic P-wave velocity  $(V_p)$ variation with time is shown in Fig. 4. Seismic velocities were obtained for each day on which a blast occurred in 76R. These velocities allowed precise (±20 feet) location of rock noises. Accuracy of seismic velocities is estimated to be  $\pm 100$  feet s<sup>-1</sup> (ref. 2). The P-wave variation with time in Fig. 4 for geophone stations 4, 5, 6 and 7 represent typical variations for all geophone locations.

Examination of Figs 2, 3 and 4 lead to the following observations. (1) The P-wave velocity increased to an apparently stable value for each geophone location once the pillar thickness was reduced to 48 feet. This result agrees with the observation that the maximum principal stress approaches a peak value once the pillar thickness is reduced to 50 feet (ref. 2). The P-wave velocity should also approach its maximum value at this time since

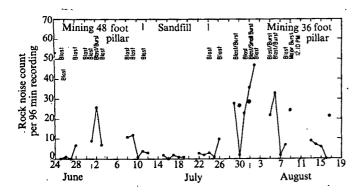


Fig. 2 Rock noise count plotted against time. (After ref. 2).

seismic velocities are known to be positively correlated with the maximum principal stress. (2) Before the major burst on August 9, the noise count in and adjacent to the pillar decreased distinctly. Noise counts resulting from the bursts of August 5 and 6 include noise counts from both zones. But when the source of each rock noise was located and separated into counts for each zone, the noise counts for the separate zones were found to decrease to low values before each of the bursts. The decrease

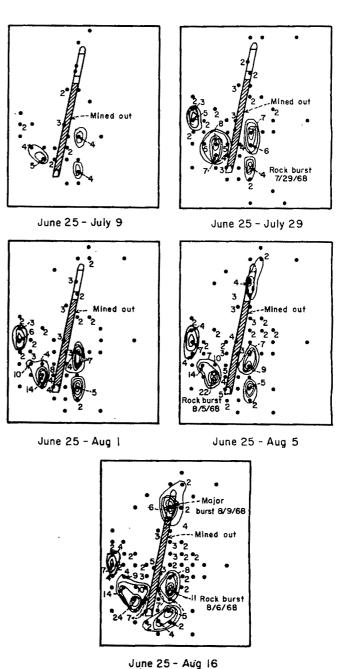


Fig. 3 Cumulative plots of rock noise source locations, 1968. (After ref. 2).

in each zone, however, was not as distinct as that observed for the major burst on August 9. No apparent decrease in noise count was observed before the small burst on August 1. These results suggest that the volume of rock involved in the burst may be an important factor in determining whether precursor phenomena, such as anomalous seismic activity, are observed before rock failures. (3)  $V_p$  decreases by approximately 6-8% before the rock bursts on August 1, 5, 6 and 9 shown in Figs 2,

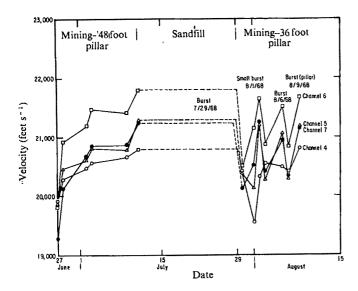


Fig. 4 P-wave variation with time and pillar geometry (76R).

3 and 4, and in general then regains its normal value, although the burst on August 6 did not occur when the velocity had returned to normal. This apparent exception may be a result of the effects produced by the burst of July 29 which occurred directly below the August 6 burst (Fig. 3). Note also that with the exception of the major burst on August 9, the other bursts occurred immediately after blasting.

The time intervals between the blasts in 76R are too long to attach much significance to most of the rock burst data in Fig, 4. It is significant, however, that the bursts of August 1 and 5 did occur when  $V_p$  had reached its normal value. The fact that these bursts occurred shortly after blasting precluded accurate determination of the time interval (precursor time) required for  $V_p$  to regain its normal value. This was not the case for the burst on August 9, which evolved independently of the possible effects produced by rapid load transferral as a result of blasting, which are reflected by the premature bursts on July 29, August 1, 5 and 6. It is important to note that before August 7, the noise count data suggest that there was a pronounced increase in stress within the pillar (Fig. 3). On August 8, however, there were few rock noises in the pillar even after blasting. The decrease in noise count in the pillar occurred at the same time that the P-wave velocity was increasing. This observation suggests that rather than releasing strain energy in the form of rock noises, the pillar had instead 'tightened up' and was storing strain energy. This effect could explain the observed increase in  $V_p$  and the decrease in the noise count before the burst. There are no data available on possible seismic activity that may have occurred in the pillar a few hours before the burst.

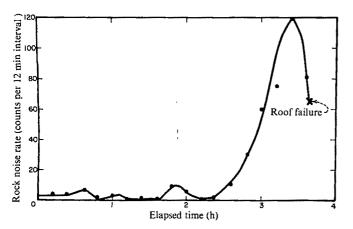


Fig. 5 Roof rock noise rate against time before roof failure.

### Coal mine roof fall

A recent application of microseismics is to provide an early warning of roof falls. The system used by the Bureau of Mines<sup>3</sup> had its first test during a controlled roof fall experiment conducted at the experimental coal mine of the United States Bureau of Mines at Bruceton, Pennsylvania. A room was first mined out and then widened in 0.31-m increments until the roof failed. The rate of emission of rock noise was monitored during mining. The results, illustrated in Fig. 5, show an anomalously high rate of rock noise emission beginning approximately 1 h before the failure. The noise count reached a maximum which

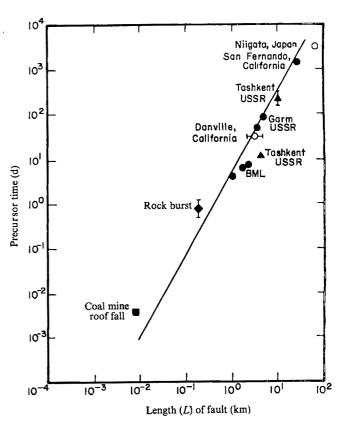


Fig. 6 Precursor time for several different failures as a function of source dimension.  $\bigcirc$ , Crystal movements;  $\blacktriangle$ , radon emission;  $\bullet$ ,  $V_p/V_a$  anomaly;  $\blacklozenge$ ,  $V_p$  anomaly;  $\blacksquare$ , noise count.

was then followed by a distinct decrease preceding the failure by about 10 to 15 min. The area of roof rock involved in the failure was approximately 70 m<sup>2</sup>.

### **Implications**

Observations at Garm (Soviet Union), the New York Adirondacks and for the San Fernando, California earthquake have shown that before these earthquakes the ratio of longitudinal  $(V_p)$  and shear  $(V_s)$  seismic velocities,  $V_p/V_s$ , decreased to anomalously low values and that earthquakes occur shortly following the return of  $V_p/V_s$  to its normal value<sup>4-7</sup>. These studies have also shown that  $V_p$  behaviour is the primary factor in the anomalous  $V_p/V_s$  behaviour. Anomalous behaviour in seismic rate (beginning before earthquakes) whose trend is qualitatively similar to that discussed earlier in mine failures has also been reported by Scholz et al. who reported that seismic activity decreased as the  $V_p/V_s$  value decreased before an earthquake of M = 2.5 in the Blue Mountain Lake region. They also observed that the seismic activity did not increase as  $V_p/V_s$ subsequently increased but decreased further; just before the earthquake the activity increased. Some investigators have explained the behaviour of these precursor effects by requiring

the diffusion of fluids into the dilatant region of an impending earthquake4,6,7.

Precursor time  $(\Delta \tau)$  versus 'effective length' (L) of the fracture area for the August 9 burst and the coal mine roof fall are listed in Fig. 6. The precursor time for the rock burst was between 1 and 2 d. The effective length for the rock burst is approximately 100 m. This estimate is based on rock source locations following the burst. The effective length for the roof fall is approximately 8 m. (Both data points lie quite close to the general trend of  $\Delta \tau$ against L observed to hold for earthquakes.) The magnitude of the rock burst of August 9 was calculated to be M = 2.5 based on data obtained from seismographs located in the mining district. The precursor time against magnitude relationship for this rock burst can be shown to follow the same relationship as that observed to hold for earthquakes<sup>6,7</sup>.

These data have two important implications. First, similar processes may be involved during failure of rock both in the mine and in the earthquake region; that is, rock failure is scale independent. Second, diffusion of fluids into the focal region of a potential earthquake may not be necessary to produce most of the precursor effects reported to occur before an earthquake since qualitatively similar precursor effects are observed in mines where no fluids are present.

I have developed a scale-independent failure theory governing the initiation and growth of shear faulting in dry rock that provides an explanation of why the precursor time-lengthmagnitude relationships hold for mine failures and earthquakes. A detailed account and implications of this theory will be published elsewhere8.9.

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# letters to nature

### Observations of the radio galaxy DA240 at 11 cm

THE radio source DA240 (refs 1 and 2) has been identified<sup>3</sup> as a 15-mag elliptical galaxy of redshift z=0.0356, placing it at a distance of 215 Mpc (assuming an Einstein-de Sitter cosmology with  $c/H_0=6,000$  Mpc). The total angular extent of the source is approximately 34', which implies a linear extent of 2.1 Mpc and makes it one of the largest systems known.

As part of a programme of source polarisation observations undertaken with the Effelsberg 100-m telescope, we have mapped the polarisation and total density distribution across DA240 at a wavelength of 11 cm with a resolution of 4.8' (Fig. 1). The mode of observation and reduction has already been described'. The map was constructed from sets of right ascension scans. They did not, however, extend far enough to the west, so the base level of each scan was determined from the eastern extremity only and no gradient of base-level along the scan was removed. The map was not made under ideal atmospheric conditions, so that the limitation to the accuracy of the unswitched total intensity output is the accuracy of base-level determination. For example, a combination of several scans with a sloping base level is evident 4' north of the map's centre at the 30 mJy level. The switched polarisation output is, in contrast, virtually unaffected by the weather, being only limited by noise at the 5 mJy level. We will discuss the three components of the source DA240 individually.

North-east component. At our resolution, the extended area is overshadowed by the strong compact component 4C56.16. The distribution of polarised flux is closely similar to the distribution of total flux over the central area of this component with a percentage polarisation close to 11% at a position angle of 63°. But on the southern edge the vectors swing around to position angles near 150° and the percentage polarisation rises to over 20%.

The percentage polarisation at 49 cm, both for the compact north-east component and the adjacent low brightness area, is 22% (ref. 3), which implies that there is little depolarisation through the source at this wavelength by differential Faraday rotation. The thermal electron density must, therefore, be very low. The differential rotation at 11 cm is 20 times less than at 49 cm but, nevertheless, the polarisation of the compact source and adjacent areas summed within our beam is only 11%. This is unlikely to be caused by differential Faraday rotation across the larger beam, either at the source, as the thermal electron densities there are so low, or in the Galaxy, as the cell size would need to be very small. Rather it must be caused by changes in the intrinsic position angle polarisation. Therefore, the magnetic field direction within the source changes significantly over distances of 1 to 2'.

Central component. At both 11 cm and 49 cm, the peak flux of the central compact component is a factor of 6 weaker than the peak flux of the north-east component. Over this wavelength range the two sources, therefore, have a similar spectrum (spectral index about -0.3), flatter than the spectrum of the more extended components. But the difference in resolution of the two maps makes accurate comparison difficult. The compact sources have, however, quite different polarisation properties, for at 11 cm the polarised intensity associated with the central source is lost in the noise, indicating a percentage polarisation of less than 4%.

South-west component. Unlike the north-east component, the extended area in the south-west is not so dominated by a compact source, and fine scale structure in the polarised radiation is more clearly visible. Although the polarised flux is smaller than in the north-east, the percentage polarisation is higher: around 20 to 30%. Along the source axis, the position angle of polarisation swings from 110° near the centre to 145° about 6' further out. Because this swing occurs over a distance of a little over one beamwidth,

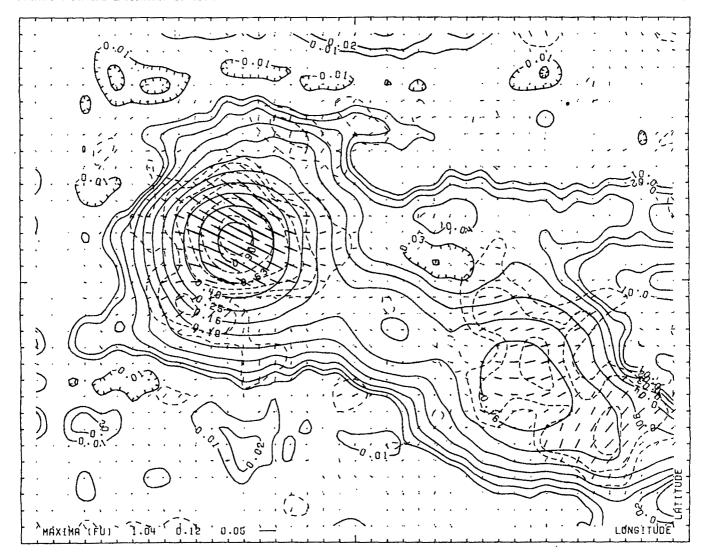


Fig. 1 DA240 at a wavelength of 11 cm. The map is centred on the position given by Bridle et al.<sup>2</sup>:  $\alpha(1950.0) = 07$  h 44 min 55 s,  $\delta(1950.0) = 55^{\circ}$  58' 43" and covers an area of 40' in  $\alpha(286$  s time at this declination) by 30' in  $\delta$ . The markers around the edge are at intervals of 1'. The full contours represent the total intensity; eleven contours labelled to an accuracy of two decimal places, are plotted with a uniform logarithmic decrement of 2 dB from (1.00 down to 0.01) times the (north-east) peak intensity places, are plotted with a uniform logarithmic decrement of 2 dB from (1.00 down to 0.01) times the (north-east) peak intensity of 1,040 mJy, except that the top contour is depressed to the 0.90 level. The dotted contours represent the linearly polarised intensity. Six unlabelled contours are plotted from (1.00 down to 0.10) times the (north-east) linearly polarised peak intensity of 120 mJy, again with the top contour depressed to the 0.90 level. The vectors have a length proportional to the polarised intensity and are oriented along the electric vector of the polarised radiation. The maxima of the total intensity and polarised intensity distributions, together with the length of the unit polarisation vector, are indicated in the lower left hand corner. Longitude and latitude correspond in this plot to  $\alpha/\delta$  (1950.0).

vectors of different orientation are present simultaneously within our beam. This will not influence the position angle of polarisation, but does yield the apparent reduction in polarised flux evident on Fig. 1 between the two areas of maximum. If the magnetic field direction in the source did not change along the axis, this swing in the 11-cm position angles would require a change in rotation measure of 50 rad m<sup>-2</sup>. Rotation measures of this order would yield high depolarisation at 49 cm, in conflict with the Westerbork observations. The rotation of the position angle of polarisation at 11 cm thus reflects a swing in magnetic field direction of  $\sim 35^{\circ}$  along the source axis over a distance of ~ 6'. Significant changes in magnetic field direction therefore seem to occur on a larger angular scale in the south-west component than in the north-east component.

Polarisation and total intensity measurements are planned at shorter wavelengths in order to delineate more precisely the changes of spectral index, rotation measure and particularly magnetic field direction across the whole of DA240.

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### A simple model of a Friedmann universe filled with a quantised scalar field

It is widely felt that the ultimate resolution of the problem of gravitational collapse awaits the formulation of a good theory of quantum gravity. Recently, however, it has been suggested (L. Parker and S. A. Fulling, unpublished) that singularities may be avoided because of quantum effects of the matter distribution rather than those of the gravitational field. When the radius of curvature of space-time becomes of the order of, say,  $10^{-13}$  cm one expects that the matter distribution might be more appropriately described by a quantum field than by its traditional description as a classical fluid. Since a quantum field need not necessarily respect the energy conditions required by the Hawking-Penrose singularity theorems, there exists, at least in principle, the possibility that in a gravitational collapse the radius of curvature of spacetime may always remain much greater than the Planck length (10-33 cm) at which quantum gravitational effects are expected to become important.

Here I present a simple model related to the occurrence of singularities in a Friedmann cosmology. The model has the virtue that analytic solutions can be obtained which should provide qualitative insight into the results that may be available from a more complete theory.

The model is a 'mini-phase space' calculation in which a massive scalar field is quantised in the background geometry of a Friedmann universe. Coordinates may be chosen so that the line element in a Friedmann space assumes the form:

$$ds^2 = dt^2 - R^2(t) \gamma_{ij} dx^i dx^j \tag{1}$$

where  $\gamma_{ij}$  is the metric of a three-space of constant curvature. The equation of motion of a scalar field in these coordinates may be found from the Lagrangian density

$$\mathscr{L} = \frac{1}{2}g^{1/2}(g^{\alpha\beta}\varphi_{,\alpha}\varphi_{,\beta} - m^2\varphi^2) \tag{2}$$

or equivalently from the action functional

$$\mathcal{S} = \int d^4x [\pi \dot{\phi} - \frac{1}{2}g^{-1/2}\pi^2 + \frac{1}{2}g^{1/2}(g^{ij}\phi,_i\phi,_j - m^2\phi^2)] \qquad (3)$$

in which  $\phi$  and  $\pi$  are to be varied independently. The energy-momentum tensor for such a field is

$$T_{\mu\nu} = 2g^{-1/2}\partial \mathcal{L}/\partial g^{\mu\nu} = \phi_{,\mu}\phi_{,\nu} - \frac{1}{2}g_{\mu\nu}(g^{\alpha\beta}\phi_{,\alpha}\phi_{,\beta} - m^2\phi^2)$$
 (4)

The problem may be simplified by restricting the phase space of the scalar field. I shall require that  $\varphi(x^t, t)$  be a function of the time t alone q(t) say.  $\pi(x^t, t)$  the momentum conjugate to  $\varphi(x^t, t)$  is a scalar density so I require it to be of the form

$$\pi(x^{i}, t) = \gamma^{1/2}(x^{i}) p(t)$$
 (5)

Equation (3) now becomes:

$$\mathcal{S} = \int d^3x \gamma^{1/2}(x^i) \int dt \{ pq - \frac{1}{2}R^{-3}p^2 - \frac{1}{2}m^2R^3q^2 \}$$

For the case in which the space is positively curved  $\int d^3x \gamma^{1/2}$  is a trivial multiplicative constant which we may drop and henceforward take:

$$\mathscr{S} = \int dt \{ pq - \frac{1}{2}R^{-3}p^2 - \frac{1}{2}m^2R^3q^2 \}$$
 (6)

In the cases of zero or negative curvature  $\int d^3x \gamma^{1/2}$  is infinite

though we may make it finite by restricting the range of the spatial coordinates and we shall adopt equation (5) in these cases also. From equation (6) we see that

$$p = R^3 \dot{q} \tag{7}$$

If we now restrict  $T_{\mu}{}^{\nu}$  to this mini-phase space, its non-zero components become

$$\rho \equiv T_0^0 = \frac{1}{2} (R^{-6} p^2 + m^2 q^2) \tag{8}$$

$$P \equiv -T_k^{\ k} = \frac{1}{2}(R^{-6}p^2 - m^2q^2) \tag{9}$$

'Canonical' commutation relationships may be imposed, namely

$$[p,q]=-i$$

The Hamiltonian is tentatively identified from equation (6) as

$$\mathcal{H} = \frac{1}{2}R^{-3}p^2 + \frac{1}{2}m^2R^3q^2$$

Defining a by

$$a = [1/\sqrt{(2m)}] [R^{-3/2}p - imR^{3/2}q]$$

we see that

$$\mathcal{H} = (m/2)(a\dagger a + aa\dagger) = m(a\dagger a + \frac{1}{2}) \tag{10}$$

We must now decide on the ordering of operators to be used in subsequent equations. This is necessary in order to determine correctly the source of the gravitational field. A natural way to do this is to order the operators so that the Hamiltonian becomes

$$\mathcal{H}_{new} = ma^{\dagger}a = \frac{1}{2} \{ R^{-3}p^2 + m^2R^3q^2 - m \}$$
 (11)

This ensures that the lowest eigenvalue of  $\mathcal{H}_{new}$  is zero, this ordering also ensures the correct ordering of the Hamiltonian in the Minkowskian regime when R/R is small.

The Schrödinger equation for the system is:

$$i\frac{\partial}{\partial t}\psi(q,t) = \mathcal{H}\psi(q,t) = \left\{ -\frac{R^3}{2}\frac{\partial^2}{\partial q^2} + \frac{m}{2}R^3q^2 - \frac{m}{2} \right\} \psi(q,t) \quad (12)$$

where the radius function R(t) may be determined from the Einstein equations

$$G_{\mu\nu} = \langle T_{new \, \mu\nu} \rangle \tag{13}$$

once the normal ordered forms of  $\rho$  and P are known. Direct calculation from equations (8) and (9) yields

$$d(\langle \rho \rangle R^3)/dt + \langle P \rangle dR^3/dt = 0$$
 (14)

But it follows from (13), by the contracted Bianchi identities that

$$d(\langle \rho_{new} \rangle R^3)/dt + \langle P_{new} \rangle dR^3/dt = 0$$
 (15)

 $\rho_{new}$  is determined from equation (11) by

$$\rho_{new} = R^{-3} \mathcal{H}_{new} = \rho - mR^{-3}/2 \tag{16}$$

Subtracting equation (15) from equation (14) then yields

$$P_{new} = P \tag{17}$$

The complete system of equations is now (henceforward all quantities are understood to be normal ordered)

$$i\partial \psi/\partial t = \frac{1}{2}(R^{-3}p^2 + m^2R^3q^2 - m)\psi$$

$$G_{\mu}{}^{\nu} = \langle T_{\mu}{}^{0} \rangle \cdot$$

$$\rho = \frac{1}{2}(R^{-6}p^2 + m^2q^2 - mR^{-3})$$

$$P = \frac{1}{2}(R^{-6}p^2 - m^2q^2)$$
(18)

We observe that although the energy density is a positive definite operator the pressure is not. Indeed the quantities  $\langle p \rangle + \langle P \rangle$  and  $\langle p \rangle + 3 \langle P \rangle$  may be negative so that the 'strong energy condition' may be violated. This violation of the energy inequalities is not directly a consequence of the normal ordering prescription, since the strong energy condition is not satisfied even by a classical massive scalar field.

The exceptional case m=0 is trivially analysed, for then  $\langle \rho \rangle = \langle P \rangle \geqslant 0$ , the conditions of the singularity theorems are satisfied and a singularity ensues.

For m > 0 it will be convenient to consider the cases k = -1, 0, +1 separately. The Einstein equations may be written in the form

$$6\ddot{R} = -(\langle \rho \rangle + 3\langle P \rangle)R$$

$$\dot{R}^2 + k = \frac{1}{3}\langle \rho \rangle R^2$$
(19)

When k=-1,  $R(t) \ge 1$  so that R(t) can have no minimum, hence R=0 at some finite time.

When k = 0,  $R(t) \ge 0$ ; if  $\dot{R}(t_0) = 0$ ,  $R(t_0) > 0$  for some  $t_0$  then

$$\langle \rho \rangle |_{t_0} = \langle P \rangle |_{t_0} = 0$$

We make the assumption that solutions of equation (18) subject to appropriate initial data are unique. Then we must have a constant solution  $R(t) = R(t_0)$ , that is, we have Minkowski space-time. The same considerations obtain also when  $t_0 = -\infty$ .

When k = +1, there exist solutions which collapse to a singularity or bounce (as we go backwards in time) depending on the initial data.

To show that a singular solution exists we may construct a self-consistent asymptotic solution to the equations (18). Near R=0 we have

$$\psi_k(q,t) \sim \exp\left\{-\frac{ik^2}{2}\int^t \frac{\mathrm{d}s}{R^3(s)} + ikq\right\}$$

with this wave function

$$\langle \rho \rangle \sim \frac{1}{2} k^2 R^{-6}, \quad \langle P \rangle \sim \frac{1}{2} k^2 R^{-6}$$

and hence by equation (19)

$$R(t) \sim [\sqrt{(3/2)kt}]^{1/3}$$

which has a zero at t = 0.

To construct a solution which has a local minimum in R(t) at t = 0 we choose as an initial state

$$|t=0\rangle = (1+\lambda^2)^{-1/2}(|0\rangle + \lambda|2\rangle)$$

where  $|0\rangle$  and  $|2\rangle$  are the eigenstates of  $\mathcal{H}(t=0)$  with eigenvalues 0 and 2m respectively. At t=0 we have

$$\langle \rho \rangle = \lambda^2 2m R^{-3}/(1+\lambda^2)$$
  $\langle P \rangle = \sqrt{2\lambda} 2m R^{-3}/(1+\lambda^2)$ 

If  $0 > \lambda > -3\sqrt{2}$  then  $\langle \rho \rangle + 3\langle P \rangle < 0$ . Choosing

$$R(0) = 2\lambda^2 m/[3(1+\lambda^2)]$$

we have  $\dot{R}(0) = 0$  and  $\ddot{R}(0) > 0$  so that R(t) has a local minimum at t = 0

From equation (18) we see that for  $R(t) \ll m^{-1}$  the equations approach, in some sense, those obtaining for zero mass for which collapse is inevitable. For any  $\varepsilon > 0$  a set of solutions of non-zero measure satisfies  $R(t) < \varepsilon m^{-1}$  for some t, and for sufficiently small  $\varepsilon$  a subset of non-zero measure of these will collapse. Thus we see that the set of solutions which exhibits a singularity is of non-zero measure.

The results of this calculation indicate that the introduction of a quantised matter source into the Einstein equations does not cause the system to 'conspire' to evade a singularity. It is of interest to note that the results of this calculation are in accord with the results obtained by L. Parker and S. A. Fulling, and W. F. Blyth and C. J. Isham, unpublished. I acknowledge fruitful conversations with Dr D. J. Raine and thank the Science Research Council for financial support.

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### Origin of fifteen cosmic dust particles intercepted by Pioneer 8 and 9

Two identical cosmic dust particle experiments on board the heliocentric space probes Pioneer 8 and 9 have now yielded consistent data for more than 8 yr. The two spacecraft are in direct heliocentric orbits with perihelia between 0.75 AU and 1.00 AU and aphelia between 1.00 AU and 1.10 AU, respectively. Their orbital velocity is approximately 30 km s<sup>-1</sup> and both satellites are spinning at 1 r.p.s. about an axis perpendicular to the ecliptic plane, and carry similar cosmic dust detectors scanning the ecliptic plane<sup>1-4</sup>. The instrumentation, its calibration, and significant scientific results have been reported<sup>1-3</sup>.

We are primarily concerned with the time-of-flight data which provided orbital characteristics of small interplanetary dust particles in the solar system. Out of the total 20 events recorded to date, 5 show hyperbolic characteristics and are excluded from this specific study. The semimajor axes and eccentricities for the remaining 15 elliptic orbits are shown in Table 1.

There are two general criteria one can apply to determine the origin of these dust particles. Whipple has introduced an empirical relationship to distinguish cometary particles from asteroid objects<sup>5</sup>, referring to it as the K criterion defined as

$$K = \log [a(1+e)/(1-e)] - 1$$
 (1)

where a is the semimajor axis in AU and e is the eccentricity. K is positive for the majority of cometary orbits and negative for the majority of asteroids. This K criterion has no basic physical significance, except as a useful tool in any discussion of the general characteristics of the meteor orbits and their origin. Computed values of K for the Pioneer 8 and 9 events are shown in Table 1. According to the K criterion, eight particles are definitely asteroidal and two are cometary. The remaining five events are intermediate and uncertain. Computed Tisserand invariant T also shows that with the exception of event 14, all of them have T greater than 1.00 and thus align with the Apollo group<sup>6</sup>. The 15 elliptical orbits have small semimajor axes, small perihelion distances, and moderate eccentricities,

Table 1 Classification of 15 cosmic dust particles detected by Pioneer 8 and 9 experiments

		Pione	er 8 and 9 experi	nents	
		Semimajor a			
Ev	ent ·	(a)	Eccentricity		
Nur	nber	(ÁÚ)	(e)	K	Type
	а	0.864	0.226	-0.86	
1	$\tilde{b}$	0.830	0.264	-0.84	Asteroidal
•	c	0.816	0.301	-0.82	Asicioladi
	a	0.623	0.729	-0.40	
2		0.601	0.729	-0.40 -0.29	Asteroidal
2	<i>b</i>	0.585	0.791		Asteroluar
	c			-0.19	
	a	0.786	0.850	0.01	T
3	b	0.812	0.905	0.21	Intermediate
	c	0.854	0.946	0.49	
	а	0.561	0.990	1.05	
4	b	0.616	0.886	-0.07	Intermediate
	c	0.763	0.624	-0.48	
	a	0.675	0.626	-0.53	
5	ь	0.652	0.685	-0.46	Asteroidal
	c	0.633	0.738	-0.38	
	a	1.010	0.090	-0.92	
6	$\vec{b}$	0.977	0.101	-0.92	Asteroidal
v	c	0.950	0.118	-0.92	ristoroldar
		0.930	0.256	-0.72	
7	a b		0.275		Asteroidal
,		0.969		-0.71	Asteroidai
	c	0.960	0.295	-0.75	
•	ą	0.830	0.544	-0.55	
8	$\boldsymbol{b}$	0.832	0.593	-0.49	Asteroidal
	c	0.830	0.639	-0.43	
	а	0.541	0.999	2.03	
10	b	0.602	0.966	0.54	Cometary
	С	0.757	0.866	0.02	
	а	1.13	0.593	-0.35	
12	b	1.16	0.645	-0.27	Asteroidal
	c	1.22	0.695	-0.17	
	a	0.555	0.993	1.45	
13	$\ddot{b}$	0.608	0.875	-0.04	Intermediate
	c	0.748	0.840	-0.07	micrinodate
	a	0.962	0.840	-0.07 -0.06	•,
14	b	1.620	0.801		Intermediate
17				0.23	intermediate
	C	5.510	0.992	2.13	
	a	0.545	0.993	1.19	<b>a</b> .
15	b	0.545	0.998	1.73	Cometary
	c	0.553	0.973	0.61	
	ď	0.719 ,	0.861	-0.02	
17	b	0,720	0.927	0.28	Intermediate
	c	0,735	0.970	0.68	
	а	0.744	0.519	-0.63	
19	b	0.712	0.586	-0.56	Asteroidal
	c	0.690	0.643	-0.40	
	-	0,070	0,015	0.10	

Listed as numbered by Berg et al.2.

Due to uncertainties in the calibration curve (typically velocities:  $\pm 18\%$ , inclinations:  $\pm 27^{\circ}$ ), three sets of possible orbital parameters are shown for each event and represented as a, b, and c. The particle mass density has been assumed to be 3 g cm<sup>-3</sup> in this analysis.

typical of the Apollo group asteroids. They cannot be the remnants of typical comets but must presumably have been produced at closer distances to the Sun.

We conclude that the majority of dust particles having elliptical orbits detected by Pioneer 8 and 9 space probes show orbital characteristics of Apollo group asteroids which originated from residual nuclei of short-period comets7.

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### Generation of magma at lunar impact crater sites

Many lunar craters have lava flows associated with them. It is widely accepted that, in most cases, the craters are the result of meteoric impact and not volcanic activity but, if so, the occurrence of lavas needs explaining. It is commonly supposed that the lava flows are material that was melted during the impact process itself but this is not acceptable as a general explanation, for two reasons. First, in several cases the ages of the lavas have been found, by crater counting techniques, to be significantly younger than the craters themselves1 and so they cannot have been formed at the time of impact. Second, some of the lavas show morphology typical of terrestrial lava flows and it is possible to deduce from this that the lava came from sources similar to those of terrestrial volcanoes—that is, lava was supplied at a fairly steady rate over periods of several days and was not simply splash-out from a meteoric impact<sup>2</sup>. It therefore seems that these lava flows are a product of the presence of an impact crater and yet have occurred as normal volcanic effusions long after the formation of the craters.

Terrestrial volcanism is strongly correlated with lithospheric plate margins. This implies that some process occurs at these margins to generate magma since there is no earthwide magma reservoir. Many mechanisms have been postulated but a likely one is that of frictional or dissipative heating due to relative motion of the plates. Shaw<sup>3</sup> showed that typical lithospheric shear stresses in systems greater than 10 km in extent would probably cause melting in the mantle, as a result of viscous dissipation, in a time of about a million years. Presumably a similar process could operate on the Moon to generate magma.

There is little evidence that there has ever been widespread motion of lithospheric plates on the Moon and it seems unlikely that this could have been the way in which magma was generated. Large scale convective motions within the Moon have been postulated but these are unlikely to penetrate nearer than 300 km to the surface4.5. Any magma generated in these motions would not be linked to the formation of impact craters except possibly the largest ones.

There is a further source of motion within the lithosphere which is associated with the formation of impact craters. It is well known that craters become shallower with age and this may be explained by a process of recovery due to viscous creep within the lithosphere. The removal of material from the surface which occurs at the formation of an impact crater produces non-hydrostatic stresses within the lithosphere which are large enough to produce a slow viscous flow. Gash<sup>6</sup> has presented a rheological model of the lithosphere which accounts for the rates of recovery of lunar craters. The model involves

Table	Recovery and melting data			
Crater diameter	Recovery time	Time to melting		
(km)	(Myr)	(Myr) 1.3		
50	21	1.3		
80	18	0.4		
130	15	0.15		
210	11	0.05		

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viscous flow at stresses greater than a yield value and much slower, though still viscous, flow at weaker stresses. The result is that craters recover relatively rapidly to a depth of about 3 km on the Moon but at a much slower rate when they are less deep. It seems possible that enough heat will be generated during the period of rapid recovery to produce magma beneath a crater. This would explain not only the production of lava at crater sites but also the time interval between the formation of a crater and the lava. Pike<sup>7</sup> discussed several ways in which lava might be generated beneath craters but found no satisfactory explanation; he preferred a process dependent on the recovery of craters to a state of isostasy. The process described here is of this kind.

Preliminary calculations have been made to assess the viability of recovery-induced melting. Gruntfest<sup>8</sup> analysed the phenomenon of dissipative heating in the presence of a temperature dependent viscosity and Shaw2 applied the results to geophysical situations. Gruntfest<sup>8</sup> showed that the effects of dissipation could be described by a parameter G which is the ratio of the rate of production of heat due to dissipation to the rate of loss of heat due to thermal conduction. For values of G less than a critical value of 0.88 in a plane shear situation the effect of dissipation is to raise the temperature slightly. After a characteristic thermal relaxation time the temperature remains steady. For values of G greater than the critical value a steady state is not achieved; eventually the temperature rises rapidly. For  $G \gtrsim 10$  the system is effectively adiabatic and the temperature theoretically becomes infinite in a finite time. In practice the increasing temperature brings about a change of phase with the result that heat is lost more rapidly, thus braking the process.

G was defined as

$$(\beta \tau^2 l^2)/\lambda \eta_0$$

where  $\tau$  is the shear stress,  $\beta$  the temperature coefficient of viscosity,  $\eta_0$  the viscosity at the initial temperature  $T_0$ ,  $\lambda$  the thermal conductivity and I the path length for thermal conduction. In the case of the recovery of lunar craters this definition needs to be modified to allow for the non-Newtonian properties of the flow. The rate of dissipation is equal to the stress multiplied by the velocity gradient,  $\nu$ . For the lunar lithosphere it is assumed, following Gash, that

$$\tau - \tau_y = \eta v$$

where  $\tau_y$  is the yield stress and  $\eta$  the viscosity so that the rate of dissipation equals

$$\tau(\tau-\tau_y)/\eta$$

This falls to zero as the stress reduces to the yield value—that is when the rapid recovery phase ends. G may be redefined, for this situation, as

$$G = \beta \tau (\tau - \tau_v) l^2 / \lambda \eta_0$$

The stress distribution beneath craters is not well known. Jeffreys<sup>9</sup> concluded that a stress of from one-half to two-thirds the surface load would exist initially beneath an excess load. For the case of a surface indentation it seems that some concentration of stress will occur<sup>6</sup> and so it is a reasonable approximation that the maximum non-hydrostatic stress beneath a crater will be equal to the maximum surface load which is  $(g\rho d)$  where d is depth of the crater and  $\rho$  the density of the lithosphere. The depth changes in time, due to the recovery process, and the change may be expressed by

$$d = d_0^{-\alpha t} \exp(-\alpha T)$$
  

$$\alpha = g \rho D_0 / 4\pi F \eta$$

 $D_0$  is the initial diameter of the crater, and F is an empirical factor introduced by Gash<sup>6</sup>. The situation is further complicated by the fact that the rate of recovery—and hence the rate at which the stress changes—is viscosity dependent.

Apart from the steady decrease in stress there are other difficulties in evaluating G for the case of lunar craters. As the temperature rises the rheology of the lithosphere will change: the yield stress will decrease and the temperature coefficient of viscosity is unlikely to remain constant. Gruntfest assumed a variation of the type

$$\eta = \eta_0 \exp(-\beta (T - T_0))$$

For a solid, the creep viscosity is more likely to follow<sup>4</sup>

$$\eta = \eta_0 T e^{E/kT},$$

where E is the activation energy for viscosity and k is Boltzmann's constant. This means that

$$\beta \sim -E/kT^2$$

and therefore varies with temperature. Tozer<sup>4</sup> predicts that E/k for the lunar lithosphere will be about 25,000 K so that  $\beta$  will range from about 0.02 just below the melting point to almost 0.3 at 300 K. Finally, the value of I is not well defined. For a crater, the depth at which maximum stress occurs is probably near to 25% of the crater diameter<sup>9</sup>. Therefore, the conduction path to the surface is probably the dominant one and I should be put equal to  $D_0/4$  although the region in which heating occurs may have a horizontal extent which is greater than this.

It follows that, for a crater,

$$G = \beta \tau (\tau - \tau_y) D_0^2 / 16 \lambda \eta_0$$

Following Gash<sup>6</sup> it is assumed that  $\eta_0 = 4 \times 10^{21} \ \text{Pa} \ \text{s}$  and  $\tau_{\nu} = 1.7 \times 10^7 \text{ N m}^{-2}$ . Before any rise in temperature  $\beta$  will be about 0.25  $K^{-1}$  and  $\lambda$  will be approximately 2 W mK<sup>-1</sup>. With these values G will be equal to its critical value at the start of the recovery process for a crater about 35 km in diameter. This is about twice the diameter of the smallest craters subject to rapid recovery<sup>6,7</sup>. This suggests that the recovery of a crater of this size may eventually produce high temperatures. It seems that the recovery of craters which are not much larger would almost certainly give rise to melting but it must be remembered that as the temperature rises both the stress and the rate of change of viscosity with the temperature decrease and so tend to reduce the rate of temperature rise. The heating will cease when the rapid recovery phase is completed and so a further requirement for melting to be achieved is that high temperatures must be produced before rapid recovery is complete.

If it is assumed that G is large enough for the process to be adiabatic then the time,  $t_{\rm ad}$ , taken for the temperature to become very large is

$$t_{ad} = \rho c \eta_0 / [\beta \tau (\tau - \tau_y)]$$

where c is the specific heat of the lunar material. The ratio M of recovery time to the time required to achieve melting reduces to

$$M = (4\pi F g \beta/c)(d_0/D_0)^2 (D_0 - D_s) \ln(D_0/D_s)$$

where  $D_s$  is the diameter of the smallest crater subject to rapid recovery and is somewhat less than 20 km on the Moon. The ratio M must be large if melting is to occur. For a 35-km diameter crater M is about 7 and so high temperatures will be produced long before recovery is complete. Some examples of characteristic recovery times for the rapid recovery phase and times needed to achieve melting are given in Table 1.

It is likely that these melting times are underestimates because of the tendency for the rate of heating to decrease as the

where

temperature rises and as recovery proceeds. It seems clear from these preliminary calculations that viscous dissipative heating could have been of major importance in the recovery of larger lunar craters. More elaborate computerised calculations may help to reveal more details of the process when more of the complexities of the situation are accounted for.

The arguments given here suggest that, for lunar craters larger than a certain size—not much greater than the minimum size of craters which undergo rapid recovery—the process of recovery gives rise to a great increase in temperature in a region beneath the crater and probably causes melting. It is hard to estimate how much melting might occur because the extent of the heated region is not known. The calculations of Gruntfest<sup>8</sup> showed that the temperature of a sheared slab does not increase equally at all points. Although the material is initially homogeneous its response to shear stress is heterogeneous and melting first occurs in a very narrow region. Furthermore, experimental work with liquids which show a marked change in viscosity at some yield stress has shown that the regions in which the lower viscosity is displayed are very narrow when the stress is of the order of the yield stress2. These regions appear as shear planes along which the strain rate is high. This evidence suggests that melting will occur in narrow zones along shear planes in the lunar lithosphere. The temperature will continue to rise and the amount of melting will increase until the system is disrupted—probably by an effusion of magma to the surface. This process may be repeated periodically. It seems that the initial melting may take up to 10<sup>7</sup> yr for the smaller craters but perhaps as little as 104 yr for the largest craters.

The depth at which melting occurs is likely to be related to crater diameter and is probably of the order of 25% of the diameter. For most craters the melting region will be within the cool lithosphere but beneath the largest craters dissipation of energy will occur in deeper material which is initially much warmer and of more basic composition than the outmost layers of the Moon. Melting will be achieved in shorter times than those given in Table 1 when the material is warmer, because of the reduced viscosity. G will remain supercritical for relatively longer in the larger systems with lower viscosity and so many more episodes of melting may occur before recovery is complete in larger craters. It would be expected that the composition of lavas would be dependent on crater diameter and this is what is generally observed. Lighter, more acidic lavas are associated with smaller craters whose sources are near the surface and dense basaltic lavas from deep in the Moon are found in the largest craters and the maria. For example, the lavas associated with the crater Tycho apparently have higher yield stresses and hence are probably more acidic than those of Mare Imbrium<sup>2</sup>.

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### Dependence of seismic mean residuals on distance, azimuth and time

Most studies on the changes of sesimic velocity which precede earthquakes (see, for example, refs 1-5) are based on data from highly sensitive, but local, seismic networks. Large earthquakes very rarely occur in the neighbourhoods of these networks, so to study velocity changes associated with earthquakes of large magnitude, it is better to use a worldwide network of stations such as those reporting to the International Seismological Centre (ISC) in Edinburgh. Studies of this kind, on the teleseismic residuals over an eight-year period at Matsushiro, have been reported by Wyss and Holcomb<sup>6</sup>. Their results show a clear

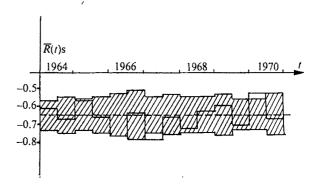


Fig. 1 The continuous line shows the mean residuals for the fourteen half-yearly periods covered by this study. ———, Overall mean residual. Hatched area denotes the 95% confidence limits for each half-yearly period, centred on the overall mean.

jump in the mean P-wave residuals in 1963, 2.5 yr before the beginning of a large earthquake swarm in the vicinity of the seismic station. Since the variation is very close to the level of statistical significance, I have made a study of similar anomalies at other stations close to the epicentres of strong earthquakes. The period covered by the data, which have been taken directly from the ISC Bulletins, is 1964-70.

Each six-month period was taken separately and all residuals under 5 s for first arrivals of P waves were used. These were divided into groups by azimuth (90° sections) and by distance (0-10°, 10°-20°, 20°-40°, 40°-60° and more than 60°), and the mean was calculated for each group (and for each six-month period and for every listed station).

Mean residuals were first calculated for seismological stations in aseismic areas in order to test whether the averaging procedure really cancels the influence of factors like differences in source mechanism, propagation path and so on. (The spatial pattern of earthquakes in different periods of time may, for instance, have a strong effect on the time variation of the mean residuals.)

As an example, the mean residuals for the Uppsala station are shown in Fig. 1. The histogram summarises the results for the 14 half-year periods. The residuals lie within  $\pm 0.14$  s of the overall mean value of -0.65 s, thus showing good stability. In two cases the observations lie outside the confidence limits, so there still remains the possibility of slight non-random variations in the mean residuals, but undoubtedly the averaging procedure is reasonably effective in suppressing irrelevant effects.

The result of -0.65 s for the overall mean residual, with a standard error of 0.01 s, was calculated from 8,553 residuals. The negative value seems to correlate with the known tendency of the Uppsala station to assign high values to the magnitudes of some seismic events. Early arrivals (negative residuals) correspond to high velocities which are normally associated with low attenuation (high Q) and, therefore, large magnitudes. Many other Scandinavian stations have negative mean residuals,

	Table 1 Dependence of	f mean residuals a	at the Uppsala stat	ion on distance an	d azimuth	
Azimuth	0°-10°	10°-20°	Distance 20°-40°	40°-60°	>60°	Total
North	$0.57 \pm 0.37 \\ 35$	$-0.50\pm0.17$	$-0.91 \pm 0.05$ $1.162$	-0.64±0.04 899	$-0.65\pm0.02$ 5,630	$-0.68 \pm 0.01$ $7.891$
East	$0.30\pm0.23$	$-1.30\pm0.42$	$0.00\pm0.22$	0	,,,,,,	$-0.14\pm0.16$
South	$-0.84\pm0.21$	$-1.94\pm0.34$	$0.24\pm0.63$	· · · · · · · · · · · · · · · · · · ·		$-1.06\pm0.21$
West	$0.07 \pm 0.37$	$-1.13 \pm 0.97$	$-0.94 \pm 0.08$	$-0.35 \pm 0.16$	0	$   \begin{array}{r}     92 \\     -0.58 \pm 0.13 \\     142   \end{array} $
Total	$-0.02\pm0.16$	$-0.80 \pm 0.14$ 224	$-0.87 \pm 0.04$ 1,299	$-0.63\pm0.04$ 921	$-0.65\pm0.02$ 5,630	$-0.65\pm0.01$ 8,553

The standard error is included with each evaluation and the number of residuals used is shown.

the magnitudes of which increase towards the centre of the Scandinavian peninsula. In North America, apart from a cluster of stations in central California with positive residuals, stations have mainly negative residuals. This too, seems to correlate with the distribution of magnitude anomalies for North American stations (A. Douglas, personal communication).

Results from many other stations in aseismic areas show the same stability. Some, however, reveal statistically significant changes in time over the period 1964–70. This would be expected in cases where changes of instrument or method of detection of first arrivals have taken place. At some stations there is a strong dependence of residuals on distance and azimuth (see, for example, Table 1).

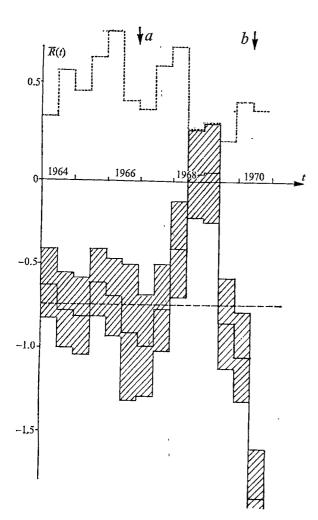


Fig. 2 Mean residulas at station Nana for the fourteen half-yearly periods with the 95% confidence limits denoted by hatching. — —, Mean residuals for station Huancayo. The arrows show the times of onset of the 1966 and 1970 Peruvian earthquakes; a, M = 7.75-8, October 1966,  $\Delta + 2.2^{\circ}$ ; b, M = 7.6, May 1970,  $\Delta, +3.4^{\circ}$ .

I now consider four sets of residuals at stations situated in the vicinity of large earthquakes which occurred in the period 1964—70. The first set is from the Nana and Huancayo stations, which are near the epicentres of the 1966 and 1970 Peruvian earthquakes. Figure 2 shows the variation of the residuals with time.

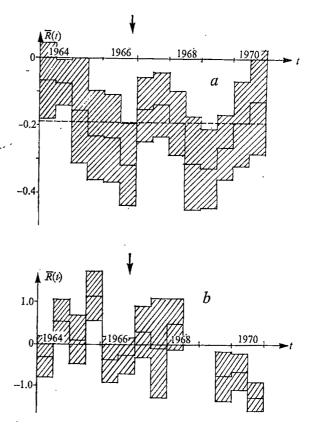


Fig. 3 Mean residuals with 95% confidence limits for Antofagasta (a) and Copiapa (b). The arrows show the time of onset of the December 1966 Chilean earthquake; a, M = 7.75-8; b, M = 7.75-8.

A statistically significant reduction in seismic velocities occurred at Nana two years before the latter event. An analogous reduction, smaller in size and occurring slightly earlier, appears for Huancayo which is a little further from the epicentre (shown without confidence limits as a dotted line in Fig. 2). On the other hand, the first earthquake is preceded by a jump in the residuals at Huancayo station only; variations at Nana before 1968 are statistically insignificant.

A second example is the Chilean earthquake of December 1966. There were two stations close to the epicentre  $(\Delta \sim 2^{\circ})$  but on almost opposite sides, Antofagasta and Copiapa. They show significant changes in mean residuals before the earthquake (see Fig. 3a and b), but they have a rather complicated structure. The pattern of a drop in velocity one or two years before the earthquake is, however, repeated at each station.

Figure 4 shows the time variation of the residuals at the Przevalsk station, 0.23° from the epicentre of a magnitude 6.8 earthquake in 1970. They follow the pattern of the Nana residuals very closely. Unfortunately, reports from the station to the ISC ceased for a 1.5-yr-period preceding the event. Significant changes in level were reported by Bullanzhe, Atrushkevich<sup>7</sup> and others in Alma Ata city, which is close to Talgar, following the earthquake.

The final example (Fig. 5) is taken from data at the Makhachkala station, situated 0.24° from the epicentre of a strong Dagestan earthquake in 1970. Because of the small number of events reported by this station, the statistics are rather poor. The pattern of variations of the mean is, however, quite similar to those of the preceding examples.

In conclusion, mean residuals of P-wave arrivals (R) show statistically significant time variations at seismic stations which are close to future earthquakes. The variations have a rather

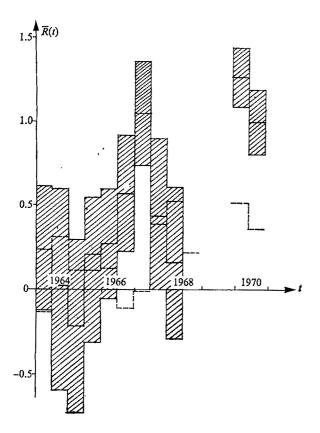


Fig. 4 Mean residuals with 95% confidence limits for Przevalsk. ———, Mean residuals for Talgar, a station situated five times further away. The arrow shows the time of onset of the June 1970 earthquake; M = 6.8.

complicated structure but the main general feature is a jump in value before the earthquake. This corresponds to a decrease in velocity and is in agreement with the predictions of the dilatancy hypothesis. The distance at which variations of R could be detected is a few times larger than the sizes of the source as determined by the aftershocks zone and by level measurements. R depends strongly on the distance and azimuth of the source. It is correlated with magnitude anomalies in a way which confirms the physical interpretation: lower attenuation corresponds to higher seismic velocities.

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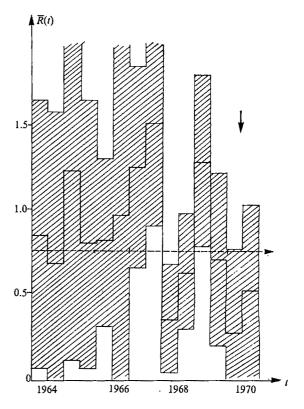


Fig. 5 Mean residuals with 95% confidence limits for Makhachkala. The arrow shows the time of onset of the May 1970 Dagestan earthquake; M=6.8.

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### Seismic slip rates in the Mediterranean and Middle East

Various plate tectonic models have been suggested for the Mediterranean and Middle East<sup>1-3</sup>. These differ in detail, but all require several smaller plates to account for the complicated seismicity observed, a simple interaction between the African, Arabian and Eurasian plates being insufficient for this purpose. It has been pointed out4,6 that recent developments in the theory of earthquake mechanism, most notably the concept of seismic moment<sup>6</sup>, enable determinations of the extent to which interactions between plates affect the rates of seismicity along their boundaries. Use of earthquake catalogues

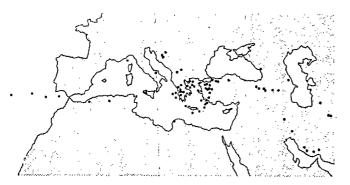


Fig. 1 Events for which seismic moment has been determined from Rayleigh wave spectral amplitudes.

for the first half of this century<sup>7.8</sup> may be combined with a magnitude-moment relation to extend the relatively short time interval for which reliable measurements of moment can be made (the last 10-15 yr), though such studies are necessarily subject to considerable error.

In spite of the large inaccuracies involved, a comparison<sup>5</sup> of relative motions between the major plates determined from seismicity over the last 70 yr with those calculated on the basis of plate tectonic theory from, for example, seafloor spreading evidence, indicated considerable agreement between the two. A notable exception to this was the western part of the Alpide Belt, where the calculated slip rate was considerably greater than that evidenced by the seismicity. In the present study accurate determinations of seismic moment have been made for all the larger ( $M_s > 5.5$ ) earthquakes in this region for the period 1963–70. These results have been used to construct a magnitude-moment relation for estimation of seismic slip between 1910 and 1970, for comparison with the rates of motion predicted by a plate tectonic model<sup>1</sup> for the region.

The latter extends from the Azores to eastern Iran (60E). The fault-plane solutions determined were used to compute the theoretical Rayleigh wave spectra for the 80 events (Fig. 1) large enough to have digitisable surface waves. The procedure adopted in the reduction of the surface-wave data is essentially that of previous workers 6.9.10. As the region studied is structurally very complex, considerable crustal and upper mantle variations will unpredictably affect the shorter periods, so observed and theoretical spectra are compared only in the period range 40–80 s

For three of the largest events, seismic moment has already been determined11,12 and these results are used here. Some of the larger shallow earthquakes in Turkey and Iran occurring before 1963 produced appreciable and well-documented surface displacements<sup>13,14</sup> from which moment may be estimated. In Fig. 2 all the moment values obtained are plotted against the corresponding surface-wave magnitude Ms. Shown on the same figure (dashed lines) are two magnitude-moment relations (B<sup>15,16</sup> and A<sup>17,18</sup>) calculated on the basis of scaling laws of the earthquake source. In the magnitude range  $6 \le M_s \le 7.5$  the relation used here is given by the solid line (C): it gives generally higher moment values than either A or B. For  $M_s > 7.5$  there is considerable disagreement between B and A as to the nature of the magnitude-moment relation: the data presented here do not convincingly favour either. Fortunately, all but one (Azores-Gibraltar region, November 25, 1941) of the higher magnitude events ( $M_s > 7.5$ ) in the area during 1910-60 were associated with surface displacements from which we may estimate moment (Fig. 2).

Relation C now permits calculation of the relative motion occurring along the major fault zones in the region considered. On the basis of the plates suggested, the Mediterranean and Middle East has been divided into 14 plate boundary 'segments' (Fig. 3 and Table 1). A search has been made through earth-quake catalogues<sup>7,19</sup> for the area and each event of  $M_s \ge 6$  assigned, wherever possible, to one of these boundary segments. For each event moment has been estimated and the moment sum for each segment calculated. We consider only the 60 yr

	Segment		Faulting		Plates	h A	obs,				$M_o^{th}/M_o^{obs}$
No.	identification	L(km)	type*	V*(cm <sub>.</sub> yr <sup>-1</sup> )		1963-70	° 1910–70	N	$\%M_{\circ}$	$M_{\rm o}^{\rm th}$	1910-70
1	Azores to 18W	900	S	0.9	AF-EU	1.2	340 (B) 16600 (A)	4 4	97	146 146	0.43(B) 0.0088(A)
2	18W to Gibraltar	1,300	T	1.1	AF-EU	770	110	6	86	515	4.7
3	Gibraltar-Tunis	1,400	Т	1.5	AF-EU	4.7	7.5	7	37	422	56
4	Sicily/Italy/Alps	2,400	M	2.0	AF-EU	13.2	105	16	34	1,270	12
5	Yugoslavia/Albania	1,000	T	. 2.0	AF-EU	26.8	6.9	10	31	528	77
6	Cretan arc	1,100	T	3.7	AF-AE	13.2	88	27	41	1,370	16
7	Northern Greece	500	N/S	2.0	EU-AE	122	98	45	10	188	1.9
8	Western Turkey	400	Ņ	3.0	AE-TU	86.3	87	24	41	241	2.8
9	Anatolian fault	1,100	S	3.2	TU-EU	222	423	23	46	352	0.83
10	South Turkey	900	T/S	3.5	AF-TU	_	1.9	3	47	865	455
11	Jordan/Syria	900	N/S	0.5	AR-AF		1.0	2	61	45	45
12	Border zone	600	S	4.2	AR-TU		3.2	-/2	88	250	78
13	E. Turkey/Caucasus	600	T	3.8	AR-EU	. 12.1	9.4	10	17	750	80
14	Iran	2,000	T	4.5	AR-EU	25.5	183	46	15	2,950	16

Segment numbers and plate names from Fig. 1. The predominant type of faulting is designated strike slip (S), thrust (T), normal (N) or mixed (M). N is the number of events for which moment has been summed and the next column gives the % of total moment contributed by the largest event.

\* From ref. 1.

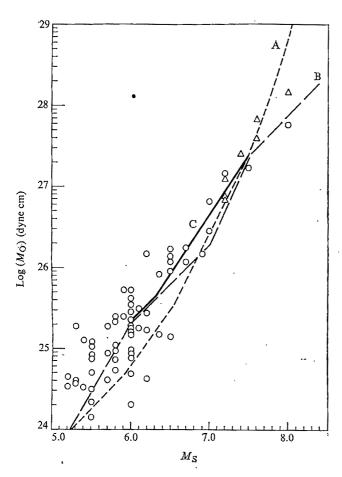


Fig. 2 Seismic moment as a function of magnitude for the events studied here (O) and for those for which moment could be estimated from surface faulting evidence (A). Also shown are the magnitude-moment relations A, B and C discussed in the text.

period 1910–70, mainly because before 1910 magnitude determinations were rarely instrumental and therefore likely to be unreliable. In Table 1 the moment sums for each segment over 1910–70 are given, together with those for 1964–70 during which moment measurements are reliable. Each has been reduced to a yearly rate and, as expected, these differ considerably, reflecting secular variations in seismicity.

For segment 1 two values are given, corresponding to a conversion of the magnitude of the large earthquake already mentioned to moment through relations B and A. The difference between the prediction of these models is very large: at  $M_s = 8.25$ ,  $M_o(A)/M_o(B) = 500$ . In Table 1 the number of events of  $M_s \ge 6$  (N) in each segment is given, together with the percentage of total moment contributed by the largest event; where this exceeds 50% the moment rate may well be a poor reflection of one based on longer term seismicity.

The rates calculated from the plate motions suggested are also given in Table 1. The relative yearly rates of motion  $(\nu)$  between these plates are obtained from the velocity vectors given and each is an average over the length L of the plate boundary segment. The depth D, to which faulting takes place is taken as 30 km except in mountainous areas (segments 5, 13, 14) where, by analogy with results for the Canoasus<sup>20</sup>, it is taken as 50 km. Seismic moment is defined as  $M_o = \mu u A$ , the product of the shear modulus  $\mu$ , (taken as  $3.3 \times 10^{11}$  dyne cm<sup>-2</sup>) the area of the faulting boundary A(=LD) and the mean relative displacement u. For a region of strike slip faulting the yearly moment rate is then  $M_o$  yr<sup>-1</sup> =  $\mu v L D$ . For areas of thrust or normal faulting, involving crustal shortening or extension,  $M_o$  yr<sup>-1</sup> =  $2\mu v L D/\sin\delta$  where  $\delta = 45^\circ$  is the average dip of the planes on which faulting takes place.

In the last column the ratio of the theoretical moment rate to the observed (equivalent to the ratio of slip rates) is given for the period 1910-70. For all but four of the segments, the percentage of moment contributed by the largest event is less than 50%, and so a ratio close to unity might be expected, on the basis of that observed elsewhere in the world<sup>5</sup>. This seems, however, to be the case only for segments 7, 8, and 9, and an alarming feature is that for no individual plate is there even reasonable agreement along all its boundaries. The best-known plate interactions, those of Africa-Eurasia and Arabia-Eurasia, show particularly poor agreement. It can reasonably be argued that observed and theoretical slip rates should be in closer agreement when the latter are large, as the seismicity should be higher and a shorter period of time sufficient. A comparison of the ratio of theoretical to observed slip rates with predicted slip rates (v) reveals that this is not the case. A curious feature is that the motions of the smaller plates such as the Aegean and Turkish, are, at least along some of their boundaries, close to those predicted.

The seismic slip rates obtained in this study are not only generally much less than those predicted by what seems to be a quite plausible model for the tectonics of this region, but are also far lower than those observed elsewhere. For the large magnitude earthquakes the moments can, with one exception, be estimated from surface faulting. In the magnitude range  $6 \le M_s \le 7.5$  the magnitude-moment relation derived predicts higher moment values than those given by relations A and B, and so these are likely, if anything, to be too high. The magnitudes themselves are unlikely to be systematically low and would need to be so by at least one unit to produce the discrepancies observed. Errors in the values chosen for the vertical extent of faulting and the shear modulus are unlikely to be more than 50%, and can hardly cause the considerable disagreement found.

The latest model<sup>1</sup> of the tectonics of this region is therefore not capable of verification by the methods described in this paper, on the basis of either present or past seismicity. No individual slip rate obtained is considered reliable enough to constitute a contradiction of the model, and in particular some of the boundaries of the minor plates are almost as active as predicted. If the earthquakes in this area are considered to result solely from an interaction between the Eurasian, African and Arabian plates, the observed seismicity rate is still an order of magnitude less than expected. None of the results obtained here contradict the existence of the smaller plates, which are in any event required to produce the complicated pattern of seismicity.

There are two possible causes for these large discrepancies. The first, and most obvious, is that 70 yr is not a long enough time for the seismic slip rates to agree with those calculated from the plate theory. If this were so, then one might expect, on the basis of the agreement found elsewhere<sup>5</sup>, that the ratios of observed to predicted slip rates would vary about unity. This is

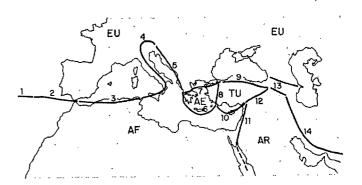


Fig. 3 Plate boundary segments to which events in this area were assigned. The plates shown are the Eurasian (EU), African (AF), Arabian (AR), Aegean (AE) and Turkish (TU).

not the case, this ratio being generally low. The most plausible explanation of the considerable discrepancies observed here is that a major proportion of the deformation in this area takes place in viscoelastic processes such as creep. This should be verifiable by the installation of strain/creepmeters in the regions where the disagreement between theory and observation is greatest.

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### Submerged platform of marine abrasion around the coasts of south-western Britain

RECENT studies at Aberystwyth have shown that Palaeozoic rocks extend as a gently sloping platform (below a cover of sediment or drift) 16 km seawards of the coast of south-western Wales, up to 18 km to the south of the coast of Ireland, and some 15 km north-west of Cornwall. Sparker traverses1 show this platform to be, in general, smoothly planed with its outer margin descending to at least 80 m. Former islands, now submerged, occur; as, for example, in St. Bride's Bay and off Tuskar Rock. Westwards of the Camel estuary the platform continues unbroken across the fault which throws Cretaceous rocks against the Palaeozoic. Shorewards no definite submerged cliff line has so far been traced and in some cases at least, the cliff line may be coincident with the outer edge of the modern coastal platform, or even the modern cliff2.

A platform of this width, cut in resistant Palaeozoic rocks, can hardly have been cut during the successive low sealeyels of the Pleistocene, which occupied in total only a small proportion of Pleistocene time. The interglacial '10 foot platform' recognised in southern and western Wales<sup>3</sup>, Devon<sup>4</sup> and southern Ireland<sup>6</sup> forms only an insignificant nick in the coastal cliffs. Moreover, this platform, probably of Cromerian age<sup>6,7</sup>, runs into already formed estuaries and bays8, and was thus preceded by a period of lower sealevel. The St Erth Beds of late Pliocene age similarly occur9,10 on the side of a pre-existing valley. The broad submerged platform of marine abrasion is, therefore older than late Pliocene.

This gives the terminus ad quem, what of the terminus a quo? The Lenham Beds of south-eastern England occur as residual deposits on the tops of hills and must precede this period of lower sealevel and prolonged erosion. It is inconceivable that deposits coeval with the Lenham Beds would not have been found in the valleys of the Weald had those valleys been in existence and submerged by the Lenham sea. Equivalents of the Lenham Beds<sup>11</sup> found in borings in the Antwerp Kampen contain Upper Miocene foraminifera, confirming an opinion previously expressed by Voorthuysen12. The cutting of the submerged platform therefore occurred between Upper Miocene and late Pliocene times.

Evidence seems to favour an end Miocene or early Pliocene date. Gignoux<sup>13</sup> has remarked on the regression of latest Miocene time, which affected western Europe. The late Miocene (Messinian) desiccation of the Mediterranean postulated by Ruggieri<sup>14</sup> has been confirmed<sup>15</sup>. This event occupied 2 Myr according to the time scale of Berggren<sup>16</sup>. The platform must have been trimmed and trimmed again during the successive regressions and transgressions of the Pleistocene but its formation occurred much earlier. Likewise, submerged river valleys crossing the shelf17 are likely to be very old, having merely been scoured out during the last regression of the sea and refilled by Flandrian deposits.

Not the least surprising feature, if this hypothesis is true, is the remarkable stability of western Britain during this long period of time, contrasting so markedly with the activity of the marginal faults during the Oligocene and Miocene. The highlevel platforms of Wales, Cornwall and Ireland must necessarily have been formed in Oligocene and Miocene times, the submerged platform being simply the last of a series of erosional features occasioned by prolonged stillstands of the sea.

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### 'Spectacle' array of <sup>210</sup>Po halo radiocentres in biotite: a nuclear geophysical enigma

POLONIUM radiohaloes occur widely and not infrequently (total about 10<sup>15</sup>–10<sup>20</sup>) in Precambrian rocks but their existence has so far defied satisfactory explanation based on accepted nucleocosmogeochemical theories<sup>1</sup>. Do Po haloes imply that unknown processes were operative during the formative period of the Earth? Is it possible that Po halos in Precambrian rocks represent extinct natural radioactivity<sup>2</sup> and are therefore of cosmological significance? A detailed comparison between an unusual array of Po halo radiocentres and U–Th halo radiocentres is presented here as bearing on the above questions.

Generally, radiohaloes occur in one of several mineralogical contexts<sup>1,3,4</sup>. First, as single haloes around discrete inclusions well isolated from other mineral defects and haloes; second, as single haloes around discrete inclusions lodged in conduits or cleavage cracks; third, as single haloes randomly spaced in clusters (sometimes overlapping); fourth, as vein haloes which formed from a continuous distribution of radioactivity (apparently deposited from hydrothermal solutions) along a conduit; and fifth, as line haloes, which surround, not conduits or cracks, but genuine single inclusions which are long (for example, 25 µm) compared with their width (perhaps 1 µm). Large, amorphous, coloured regions without discrete inclusions are not haloes.

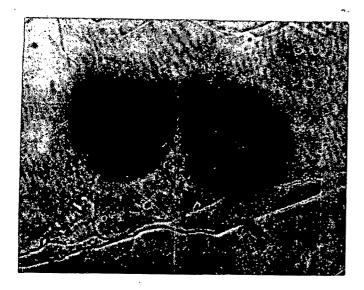


Fig. 1 'Spectacle' array of  $^{210}Po$  haloes in biotite. Halo radius,  $18.5~\mu m$ .

A striking exception<sup>1</sup> to this classification is the 'spectacle' coloration pattern (Fig. 1), which exhibits two almost circular rings of inclusions joined by a linear array of inclusions. As far as we know this is unlike any group of haloes previously seen. This geometrical arrangement of halo radiocentres, found in a Precambrian biotite from Silver Crater Mine, Faraday Township, Ontario, exhibits true radiohalo characteristics.

First, the coloration is identical to that of normal haloes found about 300  $\mu$ m away in the same mica specimen. Second, the three-dimensional nature of the halo pattern was demonstrated when the specimen (initially about 50  $\mu$ m thick) was cleaved; both halves revealed matching 'spectacle' coloration patterns, the only difference being the presence of the inclusion array in one half and its absence in the other half. Third, the radius of the coloration band (18.5  $\mu$ m) implied an origin from <sup>210</sup>Po  $\alpha$  decay. Mass spectrometric and X-ray fluorescence

methods were used to ascertain whether this was indeed a Po halo array.

Before applying these techniques to the 'spectacle' halo, we established that ion-microprobe mass analyses and scanning electron microscope X-ray fluorescence (SEMXRF) studies of 'normal' or 'standard' halo radiocentres (those formed from both U and Th  $\alpha$  decay) yielded data consistent with the visual means of identification. Several U-Th haloes (see, for example, photo insert, Fig. 2) found in a Precambrian pegmatitic mica from Rossi, New York, were analysed by X-ray and ion-probe techniques. Several U-Th halo radiocentres were chosen which contained only U, Th and Pb in any significant abundance, thereby virtually eliminating any molecular ion interference in the Pb-Th-U region (m/e = 204-238) in the ion probe.

That the mica matrix<sup>5</sup> yielded insignificant molecular ion currents in the region m/e = 160-320 is evident from the data in the lower portion of Fig. 2. In contrast, the recorded spectra of a U-Th inclusion (upper left portion of Fig. 2) revealed a significant number of ion counts accumulated in 12 passes of the regions m/e = 204-209 and (with a different scale) m/e = 232-240. Total ion counts are tabulated just above the two spectra. The scans on the Pb-Bi region (m/e = 204-209) lasted for several minutes and were taken before the scans (equal time) on the U-Th region.

Exact  $^{208}\text{Pb}/^{238}\text{U}$  and  $^{208}\text{Pb}/^{232}\text{Th}$  ratios are not obtainable from the ion count data in Fig. 2 because variable U and Th concentrations were observed as the ion probe beam sputtered away the inclusion; accurate ratios could be obtained by simultaneously accumulating counts in the region 204–238 provided that the greater secondary ion yield of U and Th as compared with Pb is taken into account. On the other hand, the separate Pb and U isotope ratios are meaningful. Note, for example, that after subtraction of background counts at m/e = 240 from the total counts at m/e = 235 and 238, the 235/238 value (0.76) satisfactorily approximates (considering the relatively small number of counts collected) the natural U isotopic ratio,  $^{235}\text{U}/^{236}\text{U} = 0.72$ . The absence of a peak at 204 shows there is little or no common lead in the inclusion and therefore, that the 206/207 ratio is that of  $^{206}\text{Pb}/^{207}\text{Pb}$  as derived from in situ U decay.

Also shown in Fig. 2 are the SEMXRF spectra of the mica matrix and the U-Th halo radiocentre, both of which correlate well (with the exception of the low Z and low abundance elements in the former) with the respective ion-probe spectra. Only U, Th and Pb are exclusively in the inclusion.

The ion-microprobe mass spectrum of the mica matrix surrounding the 'spectacle' halo was nearly identical to the mica spectrum shown in Fig. 2 and is not repeated in Fig. 3. Figure 3 (top centre) shows the portion m/e = 160-264 of the ion-microprobe spectrum (verical log scale) of several of the inclusions. Also shown is the actual ion-probe trace of the important region from m/e = 204-210 using a linear vertical scale and an expanded horizontal scale. There is no significant ion current above m/e = 209; that is, no significant ion signals were detected at any of the prominent U and Th peaks:  $238(U^+)$ ,  $254(UO^+)$ ,  $232(Th^+)$  and  $248(ThO^+)$ . No m/e = 204 was detected above background (1 c.p.s.), and the 206/207 mass ratio was  $\approx 20$  (206 signal  $\approx 2,000$  c.p.s.).

Figure 3 also shows SEMXRF spectra of the surrounding mica and of one of the Po halo radiocentres. Lead is the only element detectable in this radiocentre exclusive of the mica; some adjacent radiocentres revealed Bi as well. The use of two different instruments, and longer counting times, account for the slightly different X-ray spectra in Figs 2 and 3. The excellent resolution of the SEM showed the Pb-rich areas to coincide exactly with the Po halo radiocentres which are visible both in ordinary transmitted (Fig 1) and reflected light microscopy. Regions as close as 1 µm to the radiocentres showed virtually no Pb or Bi, implying little if any diffusion loss from the inclusions.

As the X-ray data definitely show Pb (and sometimes Bi) in the 'spectacle' halo radiocentres, and as there is no evidence for any molecular ion contribution in the region from m/e =

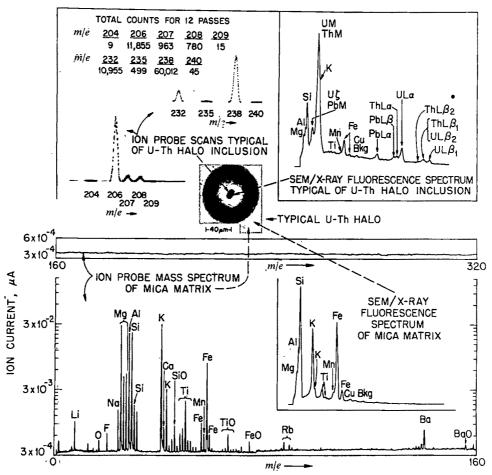


Fig. 2 Ion microprobe and XRF comparison between mica matrix and U-Th halo inclusion.

204–238, the 206, 207 and 208 peaks are interpreted as Pb isotopes and 209 as <sup>209</sup>Bi. <sup>204</sup>Pb, a constituent of both common and primordial Pb, is missing (no 204 peak), implying that the 'spectacle' halo inclusions analysed contained no detectable Pb

of either of these types. Absence of the 232, 235 and 238 peaks is interpreted as showing the inclusions contain virtually no <sup>232</sup>Th, <sup>235</sup>U or <sup>238</sup>U and, therefore, no radiogenic <sup>208</sup>Pb, <sup>207</sup>Pb or <sup>206</sup>Pb derived from the *in situ* decay of these isotopes. The 207 and 208

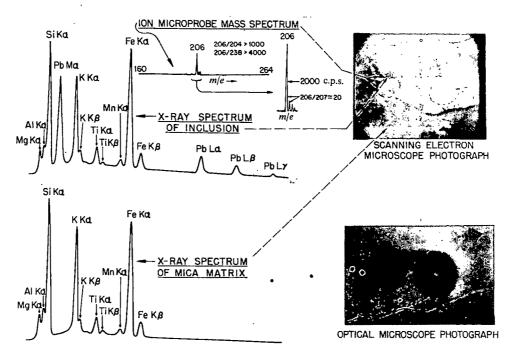


Fig. 3 Ion microprobe and SEMXRF spectra of mica matrix and 210Po halo inclusions.

peaks are therefore attributed to 207Pb and 208Pb, perhaps arising from the decay of minute amounts of 211Bi and 212Bi within the inclusions<sup>5,6</sup>. The <sup>209</sup>Bi is considered to be primordial.

The outstanding feature of the mass analysis is the prominent 206 signal which, when attributed to the presence of 206Pb in the inclusions, fits perfectly with the prediction based on ring structure measurements, that is, that the 206Pb is radiogenically derived, not from U or Th, but directly from 210Po a decay. In this respect, the large difference in the 206/238 (208Pb/238U) ratio between the 'spectacle' halo and the U-Th halo (Figs 2 and 3) is especially significant. Clearly the 'spectacle' halo resulted from <sup>210</sup>Po α decay; an explanation for its geometry is still under study.

Because the Pb isotope in these inclusions is not explicable as any combination of common, primordial, or from in situ Pb derived radiogenically in situ from U or Th, we conclude that a different type of Pb, derived from Po a decay, exists in nature. Supportive evidence comes from electron-probe and ion-probe analyses of a 218Po halo radiocentre found in a mica from the Iveland District, Norway, which yielded a 206Pb/207Pb ratio of 23. This is consistent with that expected from <sup>218</sup>Po a decay to <sup>206</sup>Pb. Such a Pb ratio is impossibly high based on normal isotopic <sup>238</sup>U/<sup>235</sup>U decay, the theoretical maximum being 21.8.

Other investigations have shown varying mixtures of Uderived and Po-derived Pb may occur in the same radiocentre. for there exists an almost continuous halo spectrum stretching from 'pure' U to 'pure' Po haloes. Only a few (<0.01) Po haloes in biotite may survive the delicate sectioning process necessary for SEM X-ray analysis.

Just as important as the existence of a new type of lead is the question of whether Po haloes which occur in a granitic or pegmatitic environment (for example, in mica, fluorite or cordierite) can be explained by accepted models of Earth history1. (R. V. G. has found other 210Po haloes that differ essentially from those in granites—unpublished information.)

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### **Gravitational driving** and energy of Adriatic tides

In small semiclosed seas, tides are excited through their open boundaries by the tides in the more extensive bodies with which they 'co-oscillate'1-6. Nevertheless the energy dissipated in such seas is not necessarily advected from and lost by, the larger system<sup>5</sup>.

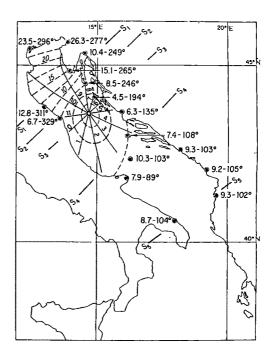
In elongated gulfs opened to the ocean, such as the Bay of Fundy and the Gulf of California, exceptionally high tides may occur. In these two cases advection of energy from the ocean largely exceeds direct gravitational forcing<sup>6,7</sup>. The opposite situation may, however, also be encountered.

Although not spectacular, the tides in the Adriatic, which at the head reach 26 cm for the main semidiurnal constituent (M2) and 18 cm for the main diurnal constituent (K1), are also remarkable in view of the modest tidal amplitudes of the adjacent Ionian Sea (6.5 cm for M2 and 1.5 cm for K1)3. Semidiurnal dissipation in the Adriatic must be low because of the effect of small amplitudes upon the faster than linear dependence of friction on velocity.

Although a square law frictional dependence must play an important role in reducing the tendency to dissipate at low amplitudes, inspection of the patterns of phases and amplitudes for M2 (cotidal and corange maps) suggests that the tidal flow is substantially in phase with the gravitational forces over most of the Adriatic. Thus, positive semidiurnal energy must be received directly from the gravitational forces balancing part or all of the frictional loss (Figs 1 and 2).

I believe that the Adriatic receives more than the energy it dissipates directly from gravitation at lunar semidiurnal frequency (M2). This conclusion is supported by calculations of energy budgets (see ref. 7) for the entire Adriatic, based on the ensemble of available observations, and a qualitative extension of considerations<sup>5</sup> about the position dependence of amphidromes (points of zero tide) upon dissipation.

Considering units of area transverse to the Adriatic, the



Cotidal and corange maps for the semidiurnal constituent M2. Observed amplitudes and phases also shown. Cotidal lines (continuous) identified according to lag in hours behind equilibrium tide on meridian 15° E, corange lines (broken) labelled in cm.

Section	Volume transport (× 10 <sup>11</sup> cm <sup>3</sup> s <sup>-1</sup> )	Phase of transport* (degrees)	Gravitational energy flux (× 10 <sup>14</sup> erg s <sup>-1</sup> )	Advection energy flux $(\times 10^{14} \text{ erg s}^{-1})$	Frictional dissipation (× 10 <sup>14</sup> erg s <sup>-1</sup> )	ţ
S <sub>1</sub> S <sub>2</sub> S <sub>3</sub>	5.1 6.4 7.2	195 194 193			•	
S <sub>4</sub> S <sub>5</sub>	5.4 1.6	197 6	1.2 to 2.45	-0.4 to -1.4	0 - 2.05	
	, F	Results for constit	uent K1, section S5	only		
S5	7.4	0-22	0.15 to -0.45	3.3 – 4.9	2.85 4.75	

<sup>\*</sup> Refers to 0000 LT.

direct gravitational energy flux,  $E^{s}$ , is the sum of individual contributions according to

$$E^{s} = \sum_{n=1}^{n} \langle T_{n} F_{n} \Delta l_{n} \rangle \tag{1}$$

where  $T_n$  is volume transport across element n, covering distances  $\Delta l_m$ ; and  $F_n$  is the force which produces the gravitational tide.

The transport  $T_n$ , or volume crossing section n in a unit time equals the amount added to the prism  $P_n$ ; the latter represents the volume of water above mean sealevel to the head of section n, with

$$P_n = \sum_{n=1}^n H_n \Delta A_n, \quad T_n = \mathrm{d}P_n/\mathrm{d}t \tag{2}$$

where  $H_n$  is tidal elevation; and  $\Delta A_n$  is the horizontal area of section n. Thus,  $T_n$ , the time derivative of  $P_n$ , is 90° ahead of  $P_n$ .

 $P_n$ . The results (Table 1) indicate that over the major part of the Adriatic T remains close to its maximum value, occurring in the amphidromic area, and that it conserves nearly the same phase  $\Phi_T$ , a lag of roughly 195° or 6.5 h. (Phases are referred to 15° E advance at an angular speed of  $\omega^{M2}=2\pi/12$  radians, or 30°, per lunar hour).

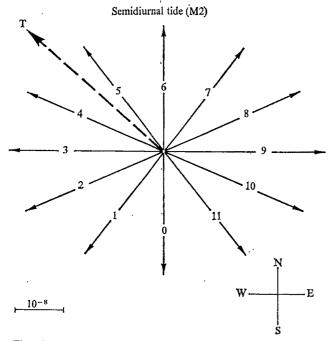


Fig. 2 Hourly variation of magnitude and geographical orientation of the Earth's surface slope with respect to equipotential surfaces for M2. T, direction of transport.

The tidal forces F (see equation 1) derive from the generating potential of the gravitational tide, W, according to

$$F = - \bar{\nabla} W \tag{3}$$

with, for the semidiurnal tide

$$W^{SD} = \rho g G^{SD} \cos^2 \theta \cos Z \tag{4}$$

where  $\overline{\nabla}$  is the horizontal gradient symbol;  $\rho$  is the specific mass of sea water; g is the acceleration of gravity;  $G^{SD}$  is the constant characteristic of each semidiurnal constituent;  $\theta$  is latitude and Z the hour angle, or longitude of lower transit of celestial body of interest—the Moon for constituent M2.

On a rigid Earth,  $G^{\text{SD}}$  for the semidiurnal constituent M2 is equal<sup>8</sup> to 24.3 cm. The elastic response of the Earth, however, depresses the tide potential by the factor (1-h+k), where h and k are the Love numbers characterising h, the solid Earth's response, and k, the resulting potential contribution<sup>9</sup>. Thus, the effective size of  $G^{\text{SD}}$  is reduced. The value (1-h+k)=0.69 has been taken, leading to  $G^{\text{SD}}=16.8$  cm for M2.

The hour-by-hour variation in magnitude and direction of the tidal slope over the Adriatic is shown in Fig. 2, together with the main geographical direction of transport—the Adriatic axis—and its phase.

Energy is supplied to the Adriatic tide when the tidal force component along the axis has the same direction as the transport. Work is done against the Moon in the opposite case. Over a tidal cycle both situations are encountered, but the gain exceeds the loss (Table 1). That can be visualised in Fig. 2.

The time-averaged influx of M2 gravitational energy is estimated at  $2.0 \times 10^{14}$  erg s<sup>-1</sup>. The main uncertainties are related to the coarseness of the integration scheme, neglect of motions transverse to the Adriatic, uncertainty on (1-h+k), and the possible influence of distortion of the Adriatic Basin through loading by the tide itself. The importance of transverse motion is commensurable with the minor-major axis ratio of the elliptical trajectories of the water particles if appropriate allowance for phase relationships is made. This all indicates an overestimation of  $E^{\mathfrak{p}}$  by a maximum of 20%. The cumulated uncertainty on the remaining factors could hardly exceed 20%. Thus, the M2 Adriatic tide receives, on an average,  $1.2-2.45 \times 10^{14}$  erg s<sup>-1</sup> directly from lunar gravitation.

Alternatively, advective driving by the Mediterranean tide is readily calculated by the flux method<sup>7</sup> according to the relationship:

$$E^{a} = \frac{1}{2} \rho g T H \cos \Phi_{T,H} \tag{5}$$

where T and H are volume transport and elevation, respective at the Adriatic-Mediterranean boundary; and  $\Phi_{T,H}$ , is the p difference between the two. Using the section between H and Durazzo, for which H is precisely known, as a boundary; and H is precisely known, as a boundary equation (2) has led to  $T = 1.6 \times 10^{11}$  cm<sup>3</sup> s<sup>-1</sup> and

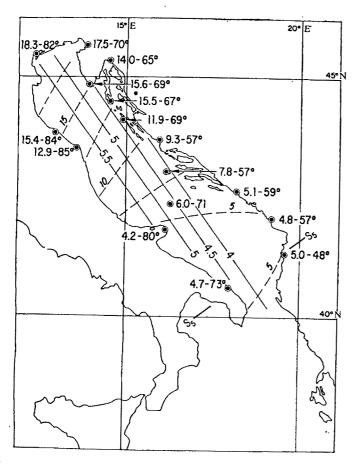


Fig. 3 Cotidal and corange map of the Adriatic for the diurnal constituent K1.

 $-98^{\circ}$ , resulting in  $E^a = -10^{14}$  erg s<sup>-1</sup>. Thus, on the average, M2 energy is advected from the Adriatic to the Mediterranean. This fact not only confirms, but indirectly requires that gravitational energy be supplied to M2 tidal motions of the Adriatic.

It should be pointed out that T and  $\Phi_{T,H}$  and, therefore, Ea, are dependent on consideration of effects from the island cluttered north-eastern shore, and on selected cotidal and corange patterns. I have, therefore, taken careful account of island areas and optimised cotidal and corange maps. Those which I selected, based on Defant's data3 (Fig. 1), do not differ significantly from those of Polli<sup>10</sup> which include more recent observations,

Estimates of tidal patterns show that displacement of the amphidrome toward the south-west reduces the energy flow to the Mediterranean. The sign changes for a 15 km displacement. Incompatibilities with the observations become noticeable, however, with a displacement of 7.5 km. Similar difficulties occur for north-eastward displacements of the order of 5 km. The energy transfer from the Mediterranean to the Adriatic thus falls between  $-0.4 \times 10^{14}$  and  $-1.4 \times 10^{14}$  erg s<sup>-1</sup>.

When the tide in a channel of constant cross-section can be represented by the sum of incident and reflected Kelvin waves with all the dissipation occurring in the reflection area, then amphidromic systems are formed with centres to the left of the axis in the northern hemisphere (the observer facing the head)5. This is valid in more general cases where dissipation also occurs along the channel. In such a case, however, the mathematical expression must differ.

In the Adriatic the evidence is for an amphidromic system very slightly displaced to the right rather than to the left (Fig. 1). This is even more evident if the north-eastern boundary is represented by a line displaced offshore from the main coastline in order to account for significant island area lost by the Adriatic Sea. Thus, here again, tidal energy loss from the Adriatic to the Mediterranean is implied.

Because conservative error bars have been accepted for E. without providing for a possibility of advective energy flow toward the Adriatic, and because independent estimates of  $E^{z}$ , which are not sensitive to small changes in cotidal and corange maps, support the same conclusion, there must be a gain of direct semidiurnal gravitational energy.

Because of the uncertainty on both  $E^a$  and  $E^g$ , frictional dissipation within the Adriatic is not well established. It may fall anywhere between zero and 2.05  $\times$  10<sup>14</sup> erg s<sup>-1</sup>.

Tidal patterns for the main diurnal constituent, K1, are proposed on Fig. 3. Hour-by-hour variation of the gravitational slope can be derived from the diurnal potential:

$$W^{D} = \rho g G^{D} \sin 2\theta \cos Z \tag{6}$$

Treatment of the K1 tide along the same lines as the M2 tide shows that direct gravitational driving,  $E^{s} = -0.3 \times 10^{14}$ erg s<sup>-1</sup>,  $\pm 50\%$ , is negative but negligible compared with the energy influx through the Mediterranean boundary,  $E^a$  $4 \times 10^{14}$  erg s<sup>-1</sup> ( $\pm 20\%$ ). Dissipation for K1 falls between 2.85 and  $4.75 \times 10^{14}$  erg s<sup>-1</sup>. Thus, it is appreciably higher than for M2, in spite of the greater amplitude and higher frequency of the

Two factors peculiar to the Adriatic play an important role in its tidal behaviour: first, the closeness of its natural resonance to tidal frequencies, and second, the phase relationship between advective and gravitational driving. The interplay between these two factors allows semidiurnal tidal currents to flow downslope with respect to equipotential surfaces most of the time, and over most of the Adriatic, permitting the tide to gain appreciable energy in spite of the small size of the gravitational forces.

The closeness of the Adriatic to resonance is dramatically illustrated by the vulnerability of Venice to flooding<sup>10,11</sup>. The Adriatic's response to storms commonly results in oscillations -seiches-with near tidal periods which, combining with the tide, create catastrophically high waters—aqua alta. The near diurnal seich, with a period of about 22 h (ref. 10) is by far the most devastating. A near semidiurnal seiche is also observed, with a period around 11 h. Most of its energy escapes, however, into the Mediterranean in much the same manner that direct gravitational energy flux at semidiurnal frequency also leaks away. Thus, the highly damped near semidiurnal seiche has less disastrous effects.

Independent verification of the importance of direct gravitational driving of the Adriatic could be made by two approaches: mathematical modelling—analytical<sup>12</sup> or digital—and experimental investigation. Sufficiently precise positioning of the amphidrome would provide a useful, though mainly qualitative, answer. There are, however, difficulties resulting from the vanishing of the signal at the point of interest13. Alternatively, precise estimates of energy exchanges between the Mediterranean and Adriatic could be obtained by adequate current measurements through the Straits of Otranto.

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### Transition to CP conservation and zero Cabibbo angle in strong magnetic fields

Most current theories of particle symmetries assume that violations of these symmetries come about through a spontaneous breaking mechanism which produces non zero expectation values for certain scalar (elementary or composite) fields. As is well known (refs 1-7 and Lebedev Institute Reprint No. 101) these expectation values may make a phase transition to a zero value for certain critical temperatures and possibly also for certain critical external magnetic field strengths  $H_c$ ,  $H_{e_1}, H_{e_2}, \ldots$  Here we point out that it is conceivable that the charge asymmetry (associated with CP violation) in  $K_L \rightarrow \pi^{\pm} +$  $l^{\pm}+\bar{\nu}(\nu)$  decays may disappear for fields of  $\sim 8\times 10^{10}$ gauss if CP violation is milli-weak in character, and that the Cabibbo angle may be reduced to zero—leading to suppression of certain hyperon decays—in fields of the order of 10<sup>16</sup> gauss. These estimates are so strongly model-dependent that it may be worthwhile in any case, to make a systematic phenomenological search for effects on particle asymmetries of strong magnetic fields of 10<sup>8</sup> gauss upwards.

The notion of critical fields (above which spontaneously broken symmetries are restored) is well known from the Ginzburg-Landau theory of superconductivity which is also the prototype of spontaneous symmetry-breaking mechanisms used in particle physics. An order of magnitude estimate of the critical field  $H_c$  in this theory is provided by considering the free energy:

$$F = F_n + \alpha(T)|\varphi|^2 + \frac{\lambda(T)}{2}|\varphi|^4 + \dots$$
 (1)

where n denotes normal.

For temperature  $T < T_c$ , F has a minimum when

$$|\langle \phi \rangle|^2 = -\alpha/\lambda \tag{2}$$

The 'thermodynamic' critical field close to  $T_c$  is then given by 8:

$$\frac{H_{c}^{2}(T)}{8\pi} = F_{n} - F_{s}$$

$$= \frac{1}{2} \frac{\alpha^{2}}{\lambda}$$

$$= \frac{1}{2} \lambda |\langle \varphi \rangle|^{4}$$
(3)

where s denotes superconducting.

Now in superconductivity theory the (Cooper-pair) field  $\varphi$  is itself charged and the magnetic field interacts directly with it. For K<sup>0</sup> fields (which we wish to investigate here) this is not the case. In an SU(3)-symmetric theory, however, the magnetic field does interact in higher order loop graphs. To see the qualitative emergence of a formula like equation (3), we later consider (for T = 0) an O(3)-symmetric gauge model, where the electromagnetic field is part of a gauge-triplet. Even though we are not dealing with a thermodynamic system, a critical field  $H_c$  seems to exist in a one-loop approximation above which the

expectation value of the neutral field  $\langle \phi_3 \rangle$  vanishes—the relationship between  $H_c$  and  $\langle \varphi_3 \rangle$  having the general form of equation (3). Here we shall use this formula for order of magnitude estimates. Clearly  $H_c$  will be small for those situations where  $\langle \varphi \rangle$  and  $\lambda$  are small.

Now for strong interaction symmetries like SU(2) or SU(3), values of  $\langle \varphi \rangle$  are typically ~ BeV •(or somewhat less), with  $\lambda \simeq 1$ . But for weak interaction symmetries—and these provide the more spectacular physical situations—with universal gauge coupling of order unity  $(g^2 \approx 4\pi/137, g \approx 1/3), \langle \phi \rangle$  values are typically tens or hundreds of BeV in magnitude, except for 'off-diagonal' situations. By way of illustration we consider the  $\langle \varphi \rangle$  associated with real rotations of the n-A system (Cabibbo angle) and ⟨φ⟩ associated with complex rotations which signal CP violation in a milli-weak manner.

To estimate the magnitude of  $H_c$  for these cases, assuming that it exists we consider the ideas of Lee<sup>9</sup>, Pais and Primack<sup>10</sup> or Mohapatra and Pati (ref. 11 and University of Maryland Technical Report No. 74-085). In their milli-weak theory, the latter authors write the mass matrix for the n, A quark system in the form:

$$f(\overline{\mathbf{n}}, \overline{\Lambda})_{\mathbf{L}} U_{\mathbf{L}} \begin{bmatrix} m_{\mathbf{n}} & 0 \\ 0 & m_{\Lambda} \end{bmatrix} V_{\mathbf{R}}^{-1} \begin{bmatrix} \mathbf{n} \\ \Lambda \end{bmatrix}_{\mathbf{R}}$$
(4)

where  $U_L$  and  $V_R$  are matrices of the type:

$$\begin{vmatrix} \cos \theta_{L,R} & -\sin \theta_{L,R} \exp(i\delta_{L,R}) \\ \sin \theta_{L,R} \exp(i\delta_{L,R}) & \cos \theta_{L,R} \end{vmatrix}$$
 (5)

with  $\theta$  and  $\delta$  real. The real part of the off-diagonal elements of the expectation-value matrix

$$U_{L} \begin{vmatrix} m_{n} & 0 \\ 0 & m_{A} \end{vmatrix} V_{R}^{-1}$$

(assuming for simplicity  $\theta_L = \theta_R$ ) is given by:

$$\frac{1}{2}(m_{\Lambda}^{2}-m_{N})\sin 2\theta_{c}\cos(\epsilon/2) \tag{6}$$

where  $\delta_L = \epsilon/2$ ,  $\delta_R = -\epsilon/2$ . Now from CP violation,  $\epsilon$  in this model can be as small as  $2 \times 10^{-3}$ . With  $m_{\Lambda} - m_{N} \simeq 175$  MeV,  $\theta_c \sim 15^\circ$  and assuming f in equation (4) to be typically  $\simeq 1$ , we obtain:

$$\text{Re}\langle \phi \rangle |_{\text{off-diagonal}} \simeq 45 \text{ MeV}$$
 (7)

Using equation (3) and making the ad hoc assumption that the numerical constant  $(\lambda)$  appearing there is of order unity, we obtain

$$H_c \simeq 3 \times 10^{16} \text{ gauss}$$
 (8)

(using the conversion  $\langle \varphi \rangle$  (MeV) = 0.3H (kgauss) cm).

If fields of this magnitude are applied, the real part of the 'off-diagonal' expectation value may vanish and, with it, the Cabibbo angle, suppressing certain hyperon and strange-particle

Also, the imaginary part of the off-diagonal matrix element of the mass matrix is

$$(f/2)(m_{\Lambda}+m_{\rm N})\sin 2\theta_{\rm c}\sin \epsilon$$

This expectation value may make a transition to a zero value for fields  $H_{\rm c} \simeq 10^{14}$  gauss—and with it the (milli-weak) •CP asymmetry—for a heavy quark model  $(m_{\Lambda} \sim 5 \text{ BeV})$ ; for a light quark model  $(m_{\Lambda} \sim 300 \text{ MeV})$ , on the other hand,  $H_c$  may reduce to 8  $\times$  10<sup>10</sup> gauss.

(A preliminary and perhaps unrealistic model for spontaneo superweak CP breaking seems to give a value of arc  $3 \times 10^6$  gauss for the critical field strength. This is likely a very gross—though morale-building—underestimate the experimental point of view.)

Since in these estimates we have assumed, for simplicity, that all model-dependent constants like f and  $\lambda$  are  $\sim$  unity and, further, since equation (3) itself is a very crude estimate, it is clear that  $H_c$  may change by orders of magnitude, and also not one but a hierarchy of critical fields may be discovered when a detailed investigation is carried through. This situation is not unfamiliar in superconductivity theory, where, depending on the ratio of the parameters  $\lambda$  and g (the gauge coupling), superconductors are divided into Type I and Type II, with Type II displaying a variety of critical fields<sup>12</sup>  $H_c$ ,  $H_{c_1}$ ,  $H_{c_2}$ ,  $H_{c_3}$  and a corresponding variety of physical (vorticity) characteristics (For V<sub>3</sub>Ga, for example, there are three widely differing critical fields,  $H_{c_1}$  (T=0)  $\simeq$  200 gauss,  $H_c$  (T=0)  $\sim$  6,000 gauss, and  $H_{c_2}$  ( $T\simeq 0$ ) which is as large as 300,000 gauss.)

Detailed models, in which we shall investigate the analogues of fields  $H_{c_1}$ ,  $H_{c_2}$ , ... for particle physics, will be considered elsewhere.

Fields of strength  $\sim 10^5$  gauss are possibly technically feasible for use in conjunction with K-beam experiments. Clearly, in order to test the ideas expressed here, stronger fields will be needed. There is no doubt, however, that if the basic ideas of spontaneous symmetry breaking are correct and if we believe in the validity of extrapolating from the one-loop calculation presented later, then there would exist critical fields for which the broken symmetries are likely to be restored. The future task of the theory is to explore such situations where the field strength required is not excessively intense and within reach of the forseeable technology. Since it is commonly assumed that the mean magnetic fields in pulsars are  $\simeq 10^{12} \simeq 10^{14}$  gauss, it would seem—for what it is worth—that CP-violating phenomena do not take place in pulsars, though the Cabibbo angle is still non-zero.

To illustrate the effect of a uniform magnetic field on symmetry breaking, we give a simple model calculation. A Lagrangian which exhibits local O(3) symmetry is given by:

$$\mathscr{L} = \frac{1}{4} F_{\mu\nu}{}^2 + \frac{1}{2} (\nabla_{\!\mu} \phi)^2 + \frac{\mu^2}{2} \phi^2 - \frac{\lambda}{4} (\phi^2)^2$$

where the scalar and vector fields,  $\phi$  and  $A_\mu,$  are triplets with respect to the O(3) symmetry and the covariant derivatives which appear are

$$\nabla_{\mu} \phi = \partial_{\mu} \phi + e \mathbf{A}_{\mu} \times \phi$$

$$\mathbf{F}_{\mu\nu} = \partial_{\mu} \mathbf{A}_{\nu} - \partial_{\nu} \mathbf{A}_{\mu} + 2e \mathbf{A}_{\mu} \times \mathbf{A}_{\nu}$$

The parameters  $\mu^2$  and  $\lambda$  are both positive: this implies a symmetry breakdown,  $O(3) \rightarrow O(2)$ , in the tree approximation. The vacuum expectation value of  $\phi$  is non-vanishing, and indeed, may be used to define a direction in iso-space

$$\langle \phi^i \rangle = \sqrt{(\mu^2/\lambda)} \delta^{13}$$
.

The corresponding component of the gauge potential,  $A_{\mu}^{3}$ , does not acquire a mass and so may be identified with the electromagnetic potential. In the tree approximation the presence of an external background electromagnetic field,  $\langle A_{\mu}^{3} \rangle \neq 0$ , has no influence on the expected value of the neutral component  $\langle \phi^{3} \rangle$  of the matter triplet.

When quantum corrections are taken into account this is no longer true. Already, the one-loop corrections to the effective action, given formally by:

$$\Gamma_{(1)}(\varphi,A) = (\hbar/2i) \operatorname{lnDet} G_{(0)}(\varphi,A)$$

(where  $G_{(0)}$  denotes the set of classical propagation function for small disturbances on a given background) imply a coupling between  $\varphi^3$  and  $A_{\mu}{}^3$  even when the charged components  $\varphi^{1,2}$  and  $A_{\mu}{}^{1,2}$  are set equal to zero in the modified ground state,

In the uniform magnetic background, where all fields vanish

except for

$$\varphi^3(x) = \psi$$
,  $A_1^3(x) = \frac{1}{2}Hx_2$ ,  $A_2^3(x) = -\frac{1}{2}Hx_1$ 

with  $\psi$  and H constant, the one-loop contribution to the effective potential,  $V_{(1)}(\psi,H)$ , is determined approximately by the differential formula

$$2\frac{\partial V_{(1)}}{\partial \psi^2} = \frac{\lambda h}{8\pi} \int_0^\infty \frac{\mathrm{d}s}{s} \left[ \left\{ \frac{eH}{\sinh(seH)} \exp\left[ -s(\lambda \psi^2 - \mu^2) \right] - \frac{\exp\left[ -s(\lambda M^2 - \mu^2) \right]}{s} \left( 1 - s\lambda(\psi^2 - M^2) \right) \right\} + \frac{3}{2\pi s} \left\{ \exp\left[ -s(3\lambda \psi^2 - \mu^2) \right] - \exp\left[ -s(3\lambda M^2 - \mu^2) \right] \times \left( 1 - 3s\lambda(\psi^2 - M^2) \right) \right\} \right]$$

(This expression is renormalised at H=0,  $\psi=M$ , that is,  $\partial V_{(1)}/\partial \psi^2$  and  $\partial^2 V_{(1)}/\partial (\psi^2)^2$  both vanish at this point.) The formula is approximate because we have not included the contributions due to intermediate vectors. The term with the H-dependent factor here is the contribution to  $V_{(1)}$  of the intermediate charge pair  $\phi^{1,2}$ , whereas the H-independent factor is due to the neutral  $\phi^3$ .

To determine the critical field, we should set  $\psi=0$  and require that  $2\partial V_{(1)}/\partial \psi^2|_{\psi=0}$  should compensate the tree contribution  $2\partial V_{(0)}/\partial \psi^2|_{\psi=0}=-\mu^2$ . Unfortunately, the neutral particle contribution to  $\partial V^{(1)}/\partial \psi^2$  is complex if  $\psi^2<\mu^2/3\lambda$ . This means that our approximation is inadequate. The source of this complexity is easily traced.

In restricting ourselves to one-loop effects we are not allowing the magnetic field to act on the neutral intermediate particles. Hence when  $\psi=0$  these appear to carry imaginary mass,  $i\mu$ . The very object of the computation  $\partial V/\partial \psi^2=0$ , is however, the setting to zero of the neutral particle mass. Clearly, we should be setting up a self-consistent (Dyson) equation: which means bringing in the contributions of higher loops. (For a similar pathology in a calculation of critical temperature see the paper of Dolan and Jackiw<sup>5</sup>.) Short of this, we can obtain an order of magnitude idea of the critical field by setting both  $\mu$  and  $\psi$  to zero specifically in the above expression for  $\partial V_{(1)}/\partial \psi^2$ . That is, we solve the restricted problem

$$0 = -\mu^{2} + \frac{\lambda h}{8\pi} \int_{0}^{\infty} \frac{\mathrm{d}s}{s} \left[ \left\{ \frac{eH_{c}}{\sinh(seH_{c})} - \frac{\exp(-s\lambda M^{2})}{s} \left(1 + s\lambda M^{2}\right) \right\} + \frac{3}{2\pi s} \left\{ 1 - \exp(-3s\lambda M^{2}) \left(1 + 3s\lambda M^{2}\right) \right\} \right]$$

or

$$\frac{\mu^2}{M^2} = \frac{\lambda^2 \hbar}{8\pi} \int_0^\infty \frac{\mathrm{d}u}{u} \left[ \frac{eH_c/\lambda M^2}{\sinh(ueH_c/\lambda M^2)} - \exp(-u) \left( \frac{1}{u} + 1 \right) + \frac{3}{2\pi} \left\{ \frac{1}{u} - \exp(-3u) \left( \frac{1}{u} + 3 \right) \right\} \right]$$
$$= \frac{\lambda^2 \hbar}{8\pi} \left[ a + b \frac{eH_c}{\lambda M^2} \right],$$

where

$$a = 1 + \frac{9}{2\pi}$$
 and  $b = \int_0^\infty \frac{d\nu}{\nu} \frac{d}{d\nu} \left( \frac{\nu}{\sinh \nu} \right)$  
$$\approx 2.5$$

On choosing the reference mass equal to  $\mu$  we obtain

$$H_{c} = \frac{1}{b} \left( \frac{8\pi}{\lambda^{2} h} - a \right) \frac{\lambda \mu^{2}}{e}$$

$$= \frac{1}{b} \left( \frac{8\pi}{\lambda^{2} h} - a \right) \frac{\lambda^{2} \langle \phi \rangle^{2}}{e}.$$

Since b is negative, we must have  $\lambda^2 H > 8\pi/a$ .

This estimate of  $H_c$  should not be taken as anything more than a rough indication. The form of this expression for  $H_c$ is basically similar to equation (3), apart from the numerical factor multiplying  $\langle \phi \rangle^2$ . It is factors of this type which in a realistic calculation may drastically alter the order of magnitude estimates given earlier. Also, for a situation involving a number of non-zero  $\langle \varphi \rangle$ s, one may conjecture that  $H_c$  would be an expression involving a sum of terms like  $\langle \Sigma f_i \phi_i \rangle^2$  with sequences of opposing signs among the coefficients  $f_i$ . This, in turn, may lead to the desired diminution of the absolute magnitudes of the critical field strengths.

It is perhaps worth remarking that the calculation presented above for the critical field does not constitute a general proof that such fields always exist. This problem clearly needs further study.

We thank Professor D. J. Bradley for pointing out that fields of 10<sup>8</sup> gauss in a small volume ~ 10<sup>-8</sup> cm<sup>3</sup> lasting more than 10<sup>-9</sup> s may conceivably be produced by laser compression techniques (though improvements in laser technology could permit the extension of both volume and duration) and to Dr Delbourgo for the remark that laser compression would also be accompanied by a high temperature which may help to produce the critical magnetic field at the same time. There is the further possibility that the electromagnetic energy pumping by the laser beam may by itself achieve the same purposes as are described in this communication. Such laser beams would have to have enormous powers of  $\sim 10^{18} \, \mathrm{W \, cm^{-2}}$ .

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### Heavy metal particle characterisation

THE aerial fallout of heavy metals downwind of the Avonmouth industrial complex has been well documented. Analyses of vegetation<sup>1</sup> and moss bags<sup>2</sup> show decreasing levels of lead, cadmium and zinc with increasing distance from the complex.

The precise nature of the heavy metal fallout, however, has been neglected although the chemical and physical state of a metal determine to a large extent its mobility in the soil and hence its availability to plants and animals.

We have, therefore, trapped air particulates and examined them by scanning electron microscopy, together with an energy dispersive Kevex X-ray analyser attached to a similar microscope. Thus it was possible to select particles down to approximately 1 µm diameter.

Air particulates were trapped on strips of adhesive aluminium tape mounted on aluminium backing plates, freely suspended at about shoulder height in the vicinity of the industrial complex, in comparable positions to those previously used1. After several weeks, 1-cm circles were cut from the tapes and mounted on electron microscope stubs. These were then vacuum coated with a thin layer of aluminium to prevent 'charging' under the microscope beam.

Debris trapped on the tapes ranged from small flies to spherical particles of less than 1 µm diameter. In particular, a large number of spherical particles (< 1 µm-10 µm) were observed under the light microscope. Because of their perfect symmetry, they were thought to be metallic droplets condensed from the vapour in furnace chimneys and flues (E. R. Buckle, and K. C. Pointon, unpublished). X-ray spectra of these particles, however, showed the majority of them to be constituted predominantly of silicon with associate sodium, potassium, calcium, iron, vanadium and titanium in varying amounts. A smaller number of particles had relatively large levels of iron. Similar spherical balls were observed on leaf samples from vineyards belonging to Long Ashton Research Station. Two apparently identical balls 3 µm diameter and about 15 µm apart on a leaf were analysed. The first gave the normal silicon type spectrum while the second showed only the Ka and KB iron peak with no other metals being identified (Fig. 1a and b).

The spectra of less regular particles were examined. Most were of the general silicon type (Fig. 1a) but a few rather more crystalline samples gave strong sulphur and calcium peaks (Fig. 1c). Others gave high sodium, chlorine or calcium peaks while one particle seemed to consist solely of sulphur. Of the three elements of most direct interest, that is, cadmium, lead and zinc, particles containing the latter were the most abundant (0.5-10 µm in diameter). These particles also contained silicon, calcium, potassium, sodium and sulphur (Fig. 1d). Other particles, however, were found to consist solely of zinc and sulphur (Fig. 1e). The Kevex energy dispersive detector does not detect oxygen which may or may not be present in these particles.

Lead occurred much less frequently than zinc, but was generally in particles in the 50-100 µm range. It was found associated with small amounts of zinc and cadmium (Fig. 1f). Cadmium was found only in isolated cases and always associated with lead.

Analyses of the tapes by acid digestion and atomic absorption spectrophotometry gave zinc/lead/cadmium ratios similar to those found in vegetation1 or moss bags2. The larger size of the lead-containing particles accounts for the much greater number of zinc particles, since the absolute amounts of the two metals is in the approximate ratio of 2 of zinc to 1 of lead.

Only a small number of the total number of particles trapped have been examined so far; hence conclusions must be tentative. Also the particles examined may not be truly representative of all fallout particles from the complex.

But these results seem to suggest the presence of zinc in • two forms: (1) in the presence of silicon, presumably as a form of silicate, and (2) in the presence of sulphur, either as sulphide or sulphate. As the Kevex detector will not respond to elements. below atomic number 11 we cannot at this stage distinguis between a sulphide and sulphate.

As lead was found in the presence of varying amoun' zinc and cadmium but not with silicon or sulphur, it m present as some form of oxide. Cadmium has been foun

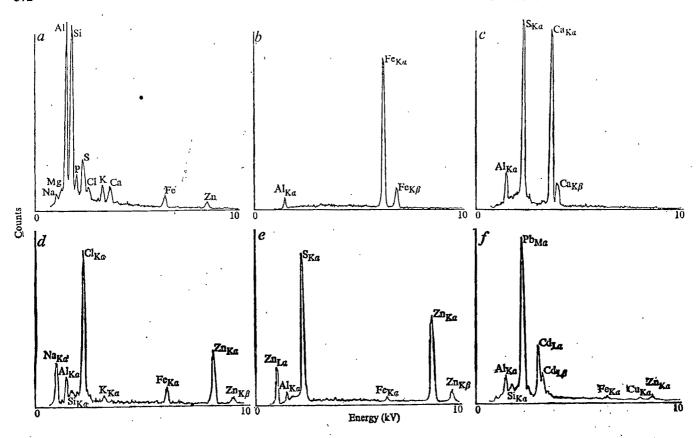


Fig. 1 X-ray emission spectra. a, Typical silicon-type particle; b, iron-containing particle; c, crystalline particle; d, zinc and silicon-containing particle; e, zinc and sulphur-containing particle; f, lead and cadmium-containing particle.

rarely and always in the presence of lead and at much lower levels.

Unfortunately, since our method is only qualitative, the relative ratios of the elements in different particles cannot be determined. Using ESCA techniques it may be possible to determine the oxidation state of the elements such as zinc, lead and sulphur within the particles. This would then offer a more meaningful indication of the exact chemical composition of the fallout. This work is now in progress.

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# Residence time of sphere or air bubble in sheared non-Newtonian fluids

During experiments to measure the residence time of air bubbles in a stirred beaker, we observed that for polyacrylamide solutions the bubbles rose more slowly as rotational speed was increased (Fig. 1). This result contradicted our expectation that 'shear thinning' of a non-Newtonian fluid would result in a more rapid rise of the bubbles. The multiple-rod impeller used in these initial experiments was similar in design to that used by Steel and Maxon¹ for aeration of a novobiocin-producing actinomycete. Single air bubbles of known volume were generated by a Chromatronix 4-way valve; bubble size could be changed by changing the size or length of the splitting loop on the valve, and the volume of the bubble was checked by measuring its length in a capillary tube of known cross-sectional area. The rise of air bubbles was traced by high speed film.

This unexpected phenomenon was further investigated in a modified Couette viscosimeter device. The bob, 3.85 cm in diameter, was driven by a variable-speed motor while the cup, 11.20 cm in diameter, was stationary. The height of solution in

Table 1 Residence time as a function of radial distance for falling

•	spl	neres			
Polymer solution	0.6%	Polyhall	1.0% Polyox		
Radial distance from bob surface (cm)	0.52	1.85	1.17	1.85	
θ (s cm <sup>-1</sup> )	0.878	0.752	0.607	0.579	
Bob rotational speed (r.p.m.) Aluminium sphere diameter (mm)	8 4.76		84 3.17		

the annular space was 21 cm. Because there was distortion of 4% and 12% on the bubble's minor and major axes as the multiple-rod impeller swept past the bubbles, rigid aluminium spheres were also used to eliminate the possibility that deformation of the bubble was a cause of the observed effect. Residence time per unit height was found by observing the time of rise or fall for six to ten repeated runs through a fixed distance of 10 cm. The relative residence time,  $\theta_{\rm r}$ , is the ratio of the residence time in a sheared solution to that in a stagnant solution. Two pseudoplastic polymer solutions were investigated: 0.6% aqueous polyacrylamide (Polyhall 295) and 1.0% aqueous polyethylene oxide (Polyox WSR-301).

When air bubbles were released at the same radial position, the relative residence time increased with increasing shear rate and eventually reached an asymptotic value for both Polyhall 295 and Polyox solutions (Figs 2 and 3). Control tests with glycerol, a Newtonian liquid, showed that  $\theta_r$  was independent of the shear rate, as it should be. These observations confirmed what had been found for the multiple-rod stirrer. On the other hand, for falling rigid spheres in the Couette device a maximum in  $\theta_r$  was noted. For small spheres at sufficiently high speeds of the bob a shear-thinning effect did occur, but at lower speeds and for larger spheres there was again an abnormally high residence time.

It is difficult to account for this behaviour. Secondary flows can occur in the annulus of a Couette device (Taylor instability), especially when the inner rather than the outer cylinder is

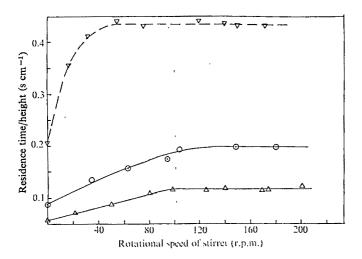


Fig.1 Residence time per unit height for air bubbles in Polyhall 295 solutions stirred by multiple-rod stirrers. The concentration of Polyhall solutions were:  $\triangle$ , 0.2%;  $\odot$ , 0.3%;  $\nabla$ , 0.5%. Air bubble diameter was 5.12 $\pm$ 0.02 mm, and the temperature of the solution was 22.5 $\pm$ 0.5° C. Bubbles were released at the mid point between the shaft and the wall.

rotated<sup>2</sup>. No such secondary flow was expected or was observed at the shearing rates used here, however, an end effect at the bottom was observed in the Polyhall 295 solution, but the torus-shaped circulation never extended above 6 cm from the bottom. The end effect did not influence the results because the timed distance for the fall of the spheres was always above this level. The anomalous shear 'thickening' effect may result from the asymmetrical flow field around a rising bubble or falling sphere; the normal stress distribution may be such as to create a net opposing elastic force. As the particle diameter increases, deviation from the ideal flow field becomes more prominent and the normal stress effect is more noticeable.

A further observation which confirms the increase in apparent transverse viscosity with increasing rate of shear is summarised

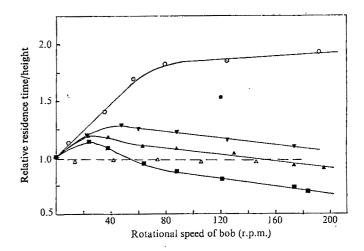


Fig. 2 Relative residence time per unit height in 0.6% Polyhall solution. ⊕, Rising air bubble in 0.6% Polyhall; △, in glycerol (0.452 N s<sup>-1</sup> m<sup>-2</sup> (452 cpoise at 22.5° C). Both at 22.5±0.2° C. Air bubble diameter was 5.13±0.02 mm. Aluminium spheres with diameters: ■, 1.59 mm; ▲,3.17 mm; ▼,4.76 mm, were released at the same radial distance from centre (r = 3.3 cm) at 25.2±0.2° C. Dashed line, Newtonian behaviour.

in Table 1. For a given size of sphere and angular velocity of the bob, the residence time increased as the radial distance between the point of release of the sphere and the bob decreased. The highest shearing rate is next to the rotating bob and, therefore, the residence time should have decreased in the vicinity of the bob if the polymer had a shear thinning property when the sphere was travelling transverse to the shear field. In qualitative terms, the chains of polymer molecules can be visualised as more uniformly aligned near the rotating bob than distant from it. Thus, a falling sphere penetrates the random molecular array at some distance from the bob faster than it penetrates the oriented or 'structured' molecules close to the rotating bob.

Highgate and Whorlow<sup>3</sup> found only slightly anomalous behaviour in a sphere falling through a sheared solution of 10% polyisobutylene in tetralin, another non-Newtonian system. They observed a decrease in 'transverse viscosity' as the shear

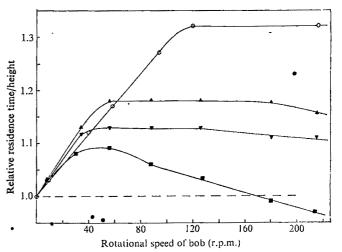


Fig. 3 Relative residence time per unit height in 1.0% Polyox WSR-301 solution.  $\odot$ , Rising air bubble with diameter  $5.13\pm0.02$  mm at  $24.7\pm0.2^{\circ}$  C. Aluminium spheres with diameters:  $\blacksquare$  1.59 mm;  $\blacksquare$ , 2.50 mm;  $\blacksquare$ , 3.17 mm, were released at the saradial distance from the centre (r=3.3 cm) at  $24.5\pm0.2^{\circ}$  Dashed line, Newtonian behaviour.

rate was increased, but the thinning effect was not as large as would be predicted from conventional measurements assuming that viscosity is an isotropic property. Perhaps still other systems and geometries need to be tested to ensure that the effect is not an artefact and to explore its dependence on the molecular properties that give rise to non-Newtonian behaviour.

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### Polymeric film precursors in the homogeneous crystallisation of some metal oxides

WE have already reported1,2 the occurrence of two-dimensional polymeric structures prior to precipitation in the system ZrOCl<sub>2</sub> (aqueous solution) -> ZrO<sub>2</sub> (monoclinic). Here we show that similar thin polymeric films are precursors in the precipitation of αFeOOH, βFeOOH, and V<sub>2</sub>O<sub>5</sub>.H<sub>2</sub>O, and that the morphology

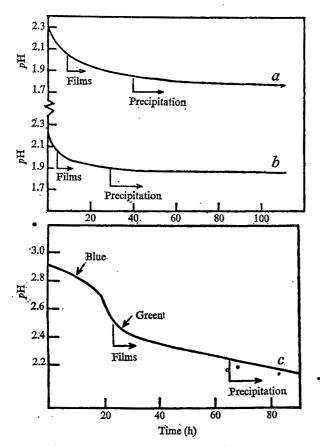
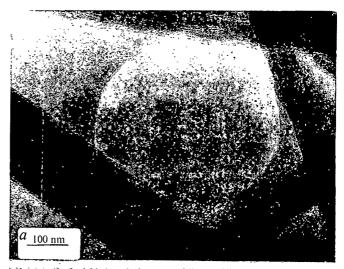
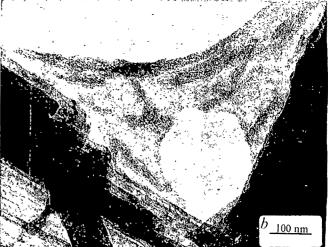


Fig. 1 Dependence of pH on time for the hydrothermal precipitation of a, a FeOOH, b, B FeOOH, c, V<sub>2</sub>O<sub>5</sub>.H<sub>2</sub>O hydrolysis at 313 K.





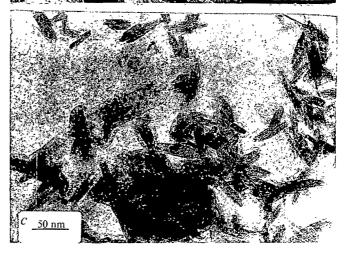


Fig. 2 a, Thin film suspended between asbestos fibres, sample taken at an early stage of film formation from FeCl<sub>3</sub>, the solution being optically clear. b, Film from FeCl<sub>3</sub> just before precipitation. The pod-like βFeOOH crystals are forming on the film the area of which is diminished as the crystals grow. c, Final precipitated product from FeCl<sub>3</sub> solution—βFeOOH.

and nature of these precipitates arises from the manner of condensation of these films to form a crystalline precipitate.

High resolution electron microscope examinations have been carried out on the aqueous systems FeCl<sub>3</sub>→βFeOOH Fe(NO<sub>3</sub>)<sub>3</sub> →αFeOOH, and VOSO<sub>4</sub>→V<sub>2</sub>O<sub>5</sub>.H<sub>2</sub>O. Also the morphology of the oxide precipitates from a range of metal oxides has been surveyed. In all cases the concentration of the solution was 0.01 M and the solution was allowed to stand at, or above, room temperature until precipitation occurred. Samples of the hydrolysis solutions were removed at intervals for electron microscopy and pH measurements. To obtain background-free micrographs and to detect film formation the aqueous samples were sprayed on to a mesh of asbestos fibres supported on an electron microscope specimen grid<sup>1</sup>.

Initially in the Fe(NO<sub>3</sub>)<sub>3</sub>, FeCl<sub>3</sub>, and VOSO<sub>4</sub> systems there was a marked fall in pH, although at this stage the samples gave no detectable deposit on the asbestos mesh. As the drop in pH diminished, examination by electron microscopy of the samples revealed thin films suspended between the asbestos fibres. The bulk solution remained optically clear during this entire period. We may conclude that the hydrolysis process was largely complete when such films had been formed in solution, since the majority of the theoretically possible fall in pH had taken place. The process of crystallisation had not begun, however, since these films showed no regular structure, nor gave rise to diffraction.

In later samples the film was observed to thicken, in some cases unevenly with regions exhibiting periodic lattice striations. This sequence of events is the same as was observed in the  $ZrOCl_2 \rightarrow ZrO_2$  system.

Figure 1 shows the pH/time dependence of the various solutions with the formation of films and precipitates indicated. Figure 2a and b show the films from the FeCl<sub>3</sub> solution with podlike structures developing in the film whilst Fig. 2c shows the

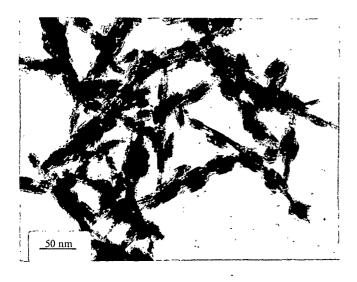


Fig. 3 The final precipitate from a FeCl<sub>3</sub>/Fe(NO<sub>3</sub>)<sub>8</sub> mixture with a Cl<sup>-</sup>/NO<sub>3</sub><sup>-</sup> ratio of 0.2. The crystals had the structure of  $\alpha$ FeOOH but are of similar morphology to  $\beta$ FeOOH, as in Fig. 2c.

final precipitated product— $\beta$ FeOOH. The VOSO<sub>4</sub> and Fe(NO<sub>3</sub>)<sub>3</sub> systems showed even film thickening and ultimately lath-shaped crystals of V<sub>2</sub>O<sub>5</sub>. H<sub>2</sub>O and  $\alpha$ FeOOH were precipitated. Similar lath-shaped crystals were observed as the final precipitates for the systems: SnCl<sub>2</sub>, SnCl<sub>4</sub> and AlCl<sub>3</sub>. NbCl<sub>5</sub> and TiCl<sub>4</sub> gave an irregular crystal and small cubic crystal respectively but in both cases hydrolysis and precipitation occurred immediately the salt was dissolved in water. To investigate the effect of the anion, mixtures of Cl<sup>-</sup> and NO<sub>3</sub><sup>-</sup> in the Fe<sup>3+</sup> solutions were hydrolysed, and these gave precipitates of the crystal structure of  $\alpha$ FeOOH for anion ratios 0–50% Cl<sup>-</sup>/NO<sub>3</sub><sup>-</sup> but the precipitate

had the pod-like shape of βFeOOH (Fig. 3). Greater Cl<sup>-</sup> proportions gave pure βFeOOH crystals and no mixtures of crystal products were observed. During crystal formation there was very little drop in pH. The similarity in appearance between the thin films of polymeric species produced in both the Fe(NO<sub>3</sub>)<sub>3</sub> and the FeCl<sub>3</sub> systems indicate that the final crystalline product is determined by the manner in which the anionic counter ion affects the film thickening process.

In these solutions both the Cl<sup>-</sup> and  $NO_3$ <sup>-</sup> are present in 0.03 M compared to an OH<sup>-</sup> concentration of  $10^{-11}$  M initially. The Cl<sup>-</sup> is a stronger complexing agent than  $NO_3$ <sup>-</sup> (ref. 4) but present in a much greater concentration than OH<sup>-</sup>, therefore, it is reasonable that the thin film would be stabilised as a two-dimensional entity by the presence of Cl<sup>-</sup> ions, in both of the axial positions of the octahedral Fe<sup>3+</sup> ions, to a greater extent than when  $NO_3$ <sup>-</sup> is a counterion.

The tendency for multilayer formation in the NO<sub>3</sub><sup>-</sup> system is, therefore, much greater, accounting for the more general thickening of the films, whilst in the Cl<sup>-</sup> system the film is more likely to crystallise by a rolling up or self condensation mechanism. The nature of the anion and its concentration, is therefore critical to these systems and a strong ligand will suppress or even stop the hydrolysis process.

These results lead to more general conclusions about precipitation mechanisms in metal oxide systems. The first stage is the hydrolysis of the metal ion which then polymerises by way of hydroxide bridges<sup>4</sup> into a two-dimensional film, with a release of hydrogen ions. The crystal formation and precipitation follows this stage with crystal growth being controlled by the anion present, which determines the manner in which the films assume a three-dimensional structure. This growth stage represents the elimination of water from hydroxide bridges to give an oxide structure. These stages may be generally represented as:

$$p[(MH_2O)_x]^{n+} \frac{\text{rapid}}{pH \text{ drop}} [M(OH)_y(H_2O)_{x-y}]_p^{p(n-y)+} + pyH^+$$
Thin film
$$Dehydration$$

$$pMO_{n/2} + (p/2)(2x-n)H_2O + pnH^+$$

where x is the coordination number of the metal ion  $M^{n+}$ , y has values  $0 \rightarrow x$ , and p is the number of molecules involved.

The OH<sup>-</sup> may be replaced by another anion which will be eliminated during the oxide lattice formation, or only partial oxide formation may occur giving rise to oxide hydroxide compounds. The pure oxide can normally be obtained by dehydration.

The pH of the solution drops to approximately 2 at the onset of film formation which would correspond to one hydrogen ion per Fe³+. Since four equatorial OH groups would, themselves, be shared between four Fe³+ ions this agrees with a monomolecular film formation in solution. The final pH of the solution depends upon the acid retention by the final oxide product. These oxides and oxide hydroxides all have a significant ion exchange capacity, and in acid solution there will be surface groups according to the equilibrium:

• 
$$M-O_2H+X^-+H_2O = MOH+H_3O^++X^-$$

It is only after prolonged washing that the anion-free precipitate is obtained. For  $\beta$ FeOOH the ion exchange capacity is 0.52 mM g<sup>-1</sup> at pH 1.35 indicating approximately one exchange able site for twenty iron atoms. This would only be possible fo very porous solid, and the regular nature of the pore struchas been reported by Gallacher<sup>5</sup>.

This uniform porosity could be obtained by the rolling up of the thin polymeric films. It is clear that crystallisation does not occur by single atom or molecule addition in any of these systems. The nature of the anion's 'complexing' ability to the metal ion relative to that of water (or OH) will determine whether a layer superimposition occurs, as in a FeOOH, or a rolling-up condensation takes place, as in βFeOOH and ZrO<sub>2</sub>, giving rise to a regular porosity.

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<sup>1</sup> Fryer, J. R., Hutchison, J. L., and Paterson, R., Nature, 226, 149

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<sup>5</sup> Gallacher, K. J., Nature, 226, 1225 (1970).

### New early Miocene vertebrate locality near Lake Rudolf, Kenya

For the past five years, Plio-Pleistocene sediments cropping out near the north-eastern shore of Lake Rudolf have been under investigation. The lacustrine and fluviatile sediments, which extend about 30 km east of the present margin of Lake Rudolf, have yielded a wealth of Plio-Pleistocene palaeontological and archaeological specimens. Provisional accounts of the geology<sup>1,2</sup>, chronostratigraphy<sup>3,4</sup>, archaeology<sup>5,6</sup>, hominids<sup>7-11</sup>, and vertebrate fossils12 have already been published.

During the summer of 1973, outcrops of Miocene sediments were discovered to the east of the Plio-Pleistocene Lake Rudolf Basin. The Miocene sediments are capped, and in many places covered, by younger igneous rocks that crop out over large portions of the region between Lakes Rudolf and Stephanie. The Plio-Pleistocene sediments are banked against the igneous rocks. Exposure of the Miocene sedimentary sequence seems on present evidence to be limited to a discontinuous belt surrounding the Suregai-Assille basalt plateau. Vertebrate fossils from this sequence were originally discovered during field investigation of the plateau basalts. Several vertebrate sites have been subsequently found on the flank of the plateau around 36° 36'E and 4° 16'N (Fig. 1).

In that region the sediments and associated volcanic rocks are folded into a series of small anticlines and synclines that trend in a NNW-SSE direction. The thickness of the sedimentary sequence is variable but at the vertebrate-bearing localities is about 50 m. There, the sediments lie on top of a deeply weathered and eroded basalt and are in turn capped by a later basalt flow. Potassium-argon age determination of the later basalt has given a date of 17.3  $\pm$  1.4 Myr (ref. 13).

The lower portion of the sedimentary sequence (Fig. 2) comprises a homogeneous and structureless red-brown clay, probably of lacustrine origin. Regression of the lake and its replacement by fluviatile and flood plain environments accounted for the subsequent superposition of a coarse silt sequence intercalated with sandstones, clays and fine silts. The most prolific vertebrate-bearing site occurred in a silt bed rich in root casts, algal structures and burrows. The bed is of limited lateral extent, seems to have accumulated in a shallow or

intermittent aquatic environment, and may best be interpreted as an interdistributary or behind-shore lagoonal facies. The upper part of the sequence was dominated by the influx of fine pyroclastic material now preserved as light coloured, reworked and crossbedded tuffs.

The fossils occur in small pockets within the coarse silt unit over a region of at least 2-3 square miles. Most of the specimens which weather out comprise rhinocerotid or proboscidean postcranial elements. The provisional faunal list is: Platybelodon kisumuensis, Prodeinotherium hobleyi, Megalohyrax championi, Dicerorhinus sp., Listriodon sp. Creodonta gen. and sp. indeterminate, Crocodylidae gen. and sp. indeterminate, Chelonia gen. and sp. indeterminate.

The presence of medium to small mammals is indicated by enamel and bone fragments which clearly do not belong to the taxa given in the faunal list. As yet, only a small part of the potentially fossiliferous sequence has received even cursory attention. All four identifiable taxa are typical of the early Miocene elsewhere in East Africa and an early Miocene age is confirmed by the radiometric date.

The fauna is not confined to the middle portion of the sequence, for Prodeinotherium hobleyi teeth have been collected subsequently from immediately above the basal basalt near the waterhole of Buluk (R. J. G. Savage, personal communication). Prodeinotherium teeth have also been found subsequently farther south near the village of Loyengalani where they were associated with a skull and mandible of Platybelodon kisumuensis (R. E. Leakey, personal communication). The strati-

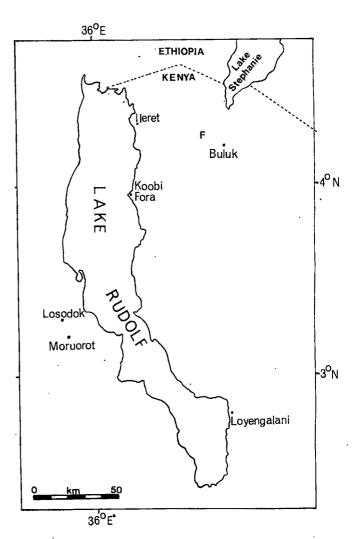


Fig. 1 Location of new Miocene locality (F).



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0.12.619150.6

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Platelets may play a major role in occlusive vascular disease, and for this reason the control of platelet function by means of inhibitors of platelet aggregation may substantially decrease the morbidity and mortality associated with these conditions. In this book there are chapters by leading investigators on the physiology and chemistry of platelet aggregation, the pharmacology of platelet aggregation inhibition and data on newly discovered inhibitors, the newer methodologic approaches to the study of platelet function, and the relationship of platelet aggregation to clinical states such as arteriosclerosis, hypertension and diabetes mellitus.

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### **Tissue Culture and Plant Science 1974**

Proceedings of the Third International Congress of Plant Tissue and Cell Culture held at the University of Leicester, Leicester, England, 21st-26th July, 1974

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0.12.673350.3

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This book provides a systematic development of geometrical optics and the optical aberration theory needed for optical design. The author provides only that material which is useful to designers with access to computers. Methods designed for outmoded computational techniques and those simply not well adapted for practical design purposes have been ignored, and the author has emphasised finite methods. This is one of the first books on aberration theory which is aimed mainly at working optical designers, and while intended primarily as a reference work, it contains some material which has previously only been published in research journals.

### The Psychology of Animal Learning

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December 1974, xxxviii+396 pp., (9" ×7", 64 plates) £8.80/\$23.25. 0.12.703950.3

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Many of the techniques described in this book have not received much attention in previous literature and so will be of particular interest; these include very high speed-flash, stereo-photography, the use of play-back tape and cave photography.

London Mathematical Society Monographs No. 4 Series Editors: P. M. Cohn and G. E. H. Reuter

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0.12.318250.6

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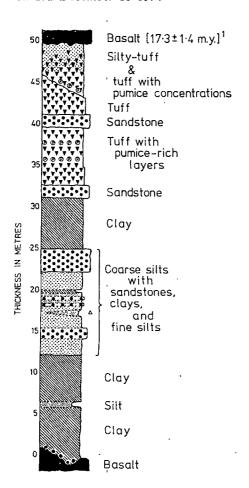


Fig. 2 Stratigraphical section at new early Miocene locality. △, Position of main vertebrate localities.

graphical relationship between the latter region and the Suregai-Assille plateau is not yet clear.

A currently more prolific early Miocene fauna has been recorded from sites of more limited lateral extent on the western side of Lake Rudolf<sup>14,15</sup>. The areal extent of the new localities on the eastern side of the lake awaits determination but is likely to prove larger than any East African site known to yield early Miocene vertebrate fossils. The new localities extend the northern limit of sub-Saharan Miocene faunas from the East African Rift System and provide complementary potential to the East Rudolf Plio-Pleistocene sediments for providing evidence of hominid origins and early evolution.

Field work was supported by grants from the Natural Environment Research Council, the National Science Foundation and the National Geographic Society. The Museum Trustees of Kenya made the fossil specimens available for study.

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### Discrimination in the assimilation of *n*-alkanes in fish

VERY large quantities of hydrocarbons have been and continue to be introduced into the seas and oceans by the various activities of man<sup>1,2</sup> but estimates of this input and comparisons with biogenically-derived material or seepage from fossil hydrocarbon deposits are difficult to make. An assessment of the general effects of non-biogenic hydrocarbons on the marine ecosystem is equally difficult.

In the analyses<sup>3</sup> we have carried out on flesh and livers from wild codling caught round the coast of the British Isles the n-alkane patterns observed fell into two general types (Fig. 1). The flesh patterns had a relatively smooth profile with a minimum around hexadecane (C16), a maximum at C26-C28 and no marked odd carbon atom predominance. The liver patterns, however, showed a marked odd carbon atom predominance, a maximum at C29-C31 and, as in the flesh, a minimum in the C16 region. These profiles bear little or no resemblance to the various alkane patterns of crude oils or indeed to most hydrocarbon products. Superficially, this suggests that the alkanes were not derived from the non-biogenic hydrocarbon input to the marine environment, but our laboratory-based studies with cod (Gadus morhua) suggest that this is not necessarily the case.

Cod liver oil capsules (containing 49 mg liver oil and 1 mg topped Kuwait crude oil) disguised in the normal squid diet were fed to codling (weight approximately 500 g) at a concentration of 1 mg crude oil d<sup>-1</sup> for 175 d. The same diet without the crude oil was fed concurrently to a control group. During the subsequent 109 d period the capsules were withdrawn from the diet. The n-alkanes content and pattern in the livers after 6 months feeding with oil showed remarkable changes (Fig. 2) in that the profile of the livers of the crude oil-fed fish lost the odd carbon atom alkane predominance and assumed a profile similar to that of the flesh (Fig. 1). The relative changes in the amount of n-alkanes are given in Table 1.

Figure 3 shows a discriminatory uptake of alkanes by the liver. Thus, the alkanes around C16 do not seem to be retained to any great extent, but as the chain length increases to about C26 the relative amount retained rises to a maximum. It is not possible to state from the results of this experiment whether these differences in the retention of the n-alkanes were due to a discriminatory absorption mechanism by the liver and/or the digestive system, and/or a preferential metabolism and/or excretion of the

Table 1 Relative changes in the amount of *n*-alkanes

. $\sum_{\text{C33}}^{\text{C15}} n\text{-alkanes (µg g}^{-1} \text{ tissue)}$								
	0	d	17	5 d	28	4 d		
Codling	Liver*	Muscle*	Liver*	Muscle*	Liver	Muscle		
Control	3.4	0.2	4.9	0.2	3.4†	0.1†		
Treated		_	29.9	0.2	13.3‡	0.2‡		

- Mean of three fish.
- Mean of four fish.
- ! Mean of five fish.

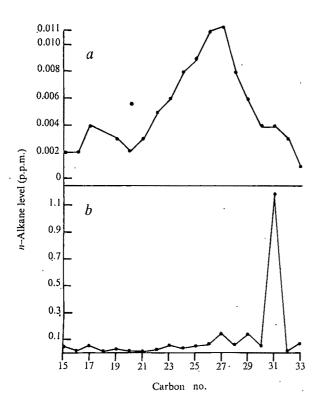


Fig. 1 Distribution of *n*-alkanes in wild codling flesh (a) and liver (b).

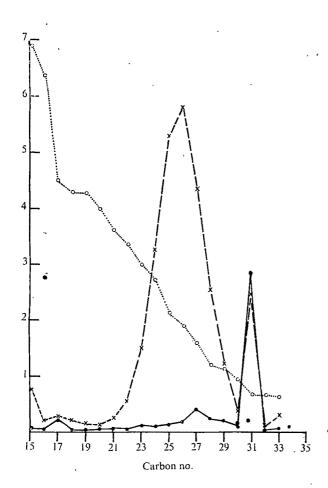


Fig. 2 Distribution of *n*-alkanes. ×, livers from codling fed crude oil for 6 months; ●, control livers (both in p.p.m.) and, ○, the crude oil used in the experiment (in µg alkanes per 100 mg crude oil). The liver results are from a mean of three fish.

shorter chain hydrocarbons. After the 6-month period following cessation of crude oil feeding, the alkanes in the liver had fallen considerably (Table 1) but the residual *n*-alkane fraction retained essentially the same oil-induced profile.

Additional experiments carried out by feeding cod with single doses of 2 µCi 1-14C hexadecane (disguised in the normal squid diet) showed that only 0.4% of the activity was recovered from the liver. Analysis of the active material by thin layer chromatography showed that it was entirely unchanged hexadecane. This seems to rule out a high metabolic conversion of hexadecane in the liver and lends some support to the presence of a mechanism in cod which discriminates between the uptake of individual *n*-alkanes in the chain length range C15-C33. This has obvious implications in considering the assimilation of hydrocarbons whether of recent biogenic or fossil fuel origin and particularly so if conclusions are drawn for the alkanes as a whole on the basis of experiments with a specific compound.

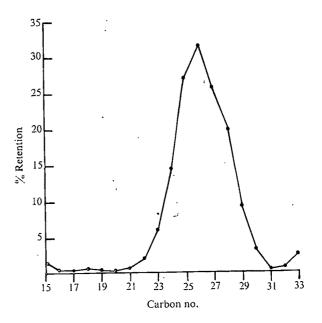


Fig. 3 Percentage retention of *n*-alkanes by the codling liver after 6 months feeding with oil as a function of carbon number.

In the cod, the liver is probably the centre of lipid metabolism and may well exercise some function of control in the deposition of alkanes in the tissues even though there is such a striking difference between the profiles of liver and flesh in normal fish. The apparent discrimination indicated by the liver analyses in the presence of a specific alkane array in the diet demonstrates clearly that, in this species, it is unlikely that a simple comparison of tissue analyses with those of the extraneous hydrocarbon source will suffice to identify the source of the tissue alkanes.

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# Possible model reactions for the nitrate reductases

NITRATE is the major source of nitrogen for most green plants and fungi and may also be used by certain microorganisms as a terminal electron acceptor in place of oxygen under anaerobic conditions. The enzymes responsible for the first step in nitrate assimilation and for nitrate respiration are the nitrate reductases, both these processes involving the conversion of nitrate to nitrite. The nitrate reductases require molybdenum both for their formation and activity<sup>1,2</sup>. Chemical tests have indicated that during the enzymatic reduction of nitrate to nitrite, the oxidation state change undergone by the molybdenum present in the enzyme from Neurospora crassa involves Mo(V) and Mo(VI)3 and ESR spectroscopic studies have strongly suggested that the nitrate is directly bound to the Mo(V) centre of the enzyme from Micrococcus denitrificans before reduction'. Therefore we have investigated whether simple molybdenum(V) complexes are able to reduce nitrate to nitrite. This has been attempted previously and either no reduction of nitrate was detected3.5 or reduction occurred to yield nitric oxide as the major product. Similarly, molybdenum-catalysed reductions of nitrate have produced at the most only traces of nitrite<sup>7-9</sup>.

We report here that oxo-molybdenum(V) complexes such as [MoOCl₃L₂], [MoOCl₄L]⁻ (where L=Ph₃PO or (Me₂N)₃PO) or [MoOCl₅]²⁻ in dichloromethane solution at room temperature, will rapidly and quantitatively convert nitrate to nitrogen(IV) oxide and slowly and quantitatively reduce nitrogen(IV) oxide to nitrogen(III), probably as NO⁺. The mechanism of the reaction between an excess (≥6:1) of Et₄NNO₃ and [MoOCl₃(OPPh₃)₂] under the above conditions has been investigated using stopped-flow kinetic techniques. The results obtained are consistent with the following mechanism:

CI 
$$Ph_3PO$$
  $Ph_3PO$   $Ph_3PO$ 

The nitrogen(IV) oxide has been removed from a 1:1 reaction mixture under reduced pressure, collected and indentified by ultraviolet and infrared spectroscopy. The complex  $[MoO_2Cl_2(OPPh_3)_2]$  has been obtained from solutions containing nitrate and molybdenum(V)  $\leq$  2:1; for the higher nitrate ratios used in the kinetic studies, the redox reaction appears to be followed by nitrate substitution at the molybdenum(VI) centre.

The labilisation of the ligand trans to an oxo group is a

well known effect<sup>10</sup> and for [MoOCl<sub>3</sub>(OPPh<sub>3</sub>)<sub>2</sub>] this results in the formation of the five-coordinate intermediate b which possesses a site available for unidentate coordination of a nitrate group to produce c. A preference for the coordination of nitrate as a bidentate ligand has been noted <sup>1</sup> and d is apparently formed by chelation which involves the displacement of a chloride ion. The geometry of d is ideal for a rapid intramolecular electron transfer from the molybdenum(V) centre to the nitrato group (Fig. 1). The typical electronic ground state for octahedral oxomolybdenum(V) complexes seems (ref. 12 and C. D. Garner, P. Lambert, and F. E. Mabbs¹, unpublished) to be  $4d_{-y}$ ).

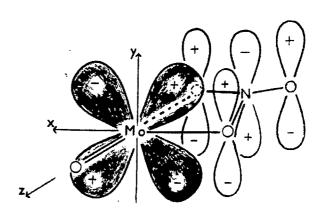


Fig. 1 Coordination of nitrate group to oxo-molybdenum (V) centre.

Nitrato complexes which contain transition metal ions in high oxidation states are powerful oxidants11 and ab initio molecular orbital calculations (I. H. Hillier, private communication) suggest that this is a result of the considerable lowering in the energy of the  $\pi^*$  orbital of the nitrate group on coordination. A rapid electron transfer from the molybdenum(V) centre to the nitrato group requires13.14 that the metal's  $4d_{xy}$  orbital overlaps to some extent with the  $\pi^*$  orbital of the nitrato group. Such overlap is only possible when the nitrato group has one and only one oxygen atom coordinated cis to the oxo group. Following the redox reaction, the electron transfer  $\pi^* \rightarrow \sigma^*$  within the nitrato group and the (slight) atomic rearrangements necessary would be expected to proceed rapidly to produce e, involving the expected10 cis- MoVIO2 unit and nitrogen(IV) oxide. As the latter has no known tendency to form complexes it is presumably readily displaced by the free triphenylphosphine oxide. At initial molybdenum(V) and nitrate concentrations of around  $1.5 \times 10^{-4}$  and  $2.5 \times 10^{-3}$ mol 1<sup>-1</sup>, respectively, this reaction sequence is completed in < 15 s at 25° C. The addition of water to the reaction mixture at this stage results in the disproportionation of the nitrogen(IV) oxide into nitrate and nitrite<sup>s 9</sup> amount of the latter being measured colorimetrically15.

The results suggest some criteria which may be important concerning the nature of the molybdenum(V) centre in the nitrate reductases. Coordination of nitrate to the metal centre could be important in lowering the activation energy for electron transfer to this group. A non-aqueous environment would favour this coordination<sup>11</sup> and therefore we suggest that the molybdenum centre of the nitrate reductases is in a hydrophobic region of the protein (which probably plays an important role in introducing the substrate to the coordination sphere of the metal). An enzymatic redox reaction between molybdenum(V) and nitrate producing molybdenum(VI) and nitrogen(IV) oxide has two main attractions: not only would the reduced species be readily displaced from the metal centre but also, once

in contact with water out of the vicinity of the metal centre, it would yield nitrite and nitrate ions, thus giving the appearance that the enzyme is actually converting nitrate to nitrite directly. The detection of nitrogen(IV) oxide in the natural systems will be difficult. ESR spectroscopy could be used, although the signal obtained from this free radical in solution can be broad and ill-defined16. The presence of an oxo group on the molybdenum(V) centre would be expected on chemical grounds10 and this group would probably dominate the reaction pathway in two respects. First, in aiding initial substrate binding trans to the oxo group<sup>17</sup> and second in controlling the electronic structure at the metal. Facile electron transfer from an oxomolybdenum(V) centre to a nitrate group will only occur if the latter is able to coordinate by way of one oxygen atom at a site cis to the oxo group with the plane of the NO<sub>3</sub> moiety containing the Mo=O axis (Fig. 1). Therefore, we suggest that, after the initial binding of the nitrate group, the enzymes must ensure that either suitable chelation or ligand rearrangement of this substrate is possible to enable one (and only one) of the oxygen atoms to be located cis to the oxo group. As there is strong evidence in favour of a molybdenum cofactor common to all the molybdenum enzymes<sup>18</sup> we suggest that the hydrophobic nature of the metal site and the facility for substrate binding cis to an oxo-molybdenum group may be important to the function of all the enzymes of this class.

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### Lack of effect of Avena sativa on cigarette smoking

AFTER his conclusion that an alcoholic extract of Avena sativa (the common oat) reduced craving for and consumption of cigarettes, Anand<sup>1</sup> recommended further investigation of its significance and role. We attempted the former by repeating his study in a situation less likely to prejudice participants than a chest ward and the latter by using a study design which would have cast light on the role of the treatment in altering smoking if such an effect had been evident.

The botanical state of his starting material was variously described as "selected just before harvest" and presumably yellow, and "ripe and green"2,3. Thus contents of the putative active ingredient could differ. We therefore studied both a green plant with full sized ears and a ripe plant dug up one week before the main harvesting. Ethanolic extracts of the plant including its roots were made exactly as described by Anand<sup>1</sup>. In preliminary blind studies the extract was nearly always distinguished from dummy despite the resources of a pharmaceutical laboratory. Therefore, two differently tasting liquids though approximating the musty quality of the oat infusion were prepared with an identical alcohol content. All three were coloured deep red. Subjects knew two dummies were included in varying order so they could not conclude from a change in taste that a period of test material had begun.

Employees of The Wellcome Foundation, Crewe, volunteered in response to a notice requesting help from smokers who would not object if their smoking habits altered while testing the effect of a new drug on smoking. No direct appeal was made to those anxious to stop smoking. At the end of the study a reward of 50p was offered. Smokers bought their own cigarettes.

Subjects had diary sheets and recorded their daily cigarette consumption by joining up any two dots randomly from an array of dots for each cigarette, thereby hindering a too ready comparison of their daily consumption. They also recorded the doses of extract taken each day, a subjective enjoyment rating of the day's cigarettes (better, same, less, none), any unwanted symptoms or effects as, for example, those brought on by a cold and their opinion as to whether they had received dummy or oat extract. They knew no treatment would last for less than one week and that the first would be a dummy to provide a run-in period. Diary pages were collected from the subjects separately each week and the next week's liquid issued in exchange for the used bottle. Forty-three subjects participated in the first study and eighteen of them continued into the second

Treatments were of 1 ml of appropriate ethanolic liquid diluted with 4 ml of water for palatability and taken four times daily during waking hours. They were presented as shown in

Study 1	Extrac	t immature oa	ts (EIO) v dun	nmy (D)			
week	Group 1	Group 2	Group 3	Group 4			
1	D1	D2	D2	D1			
2	D2	D1	D1	$\mathbf{D2}$			
3–6	EIO	EIO	D2	<b>D</b> 1			
7-10	D1	D2	EIO	EIO			
11–12	No treatment						
No. of	_		_				
subjects	7	14	7	15			

Volunteers had to decide whether they had taken oats or dummy infusion. Statistical analysis suggested that their decisions were guesses rather than detections of the oat preparation. Any persistent effects would have been observed in the period following the oats administration. The volunteers expected to learn at the end of the tenth week in which period

they took the test liquid. Actually all were misinformed and only correctly informed at the end of the twelfth week. If any real effect had operated by psychological rather than pharmacological means it might have diminished in volunteers of groups 3 and 4 during weeks 11 and 12.

Subjects' cigarette consumptions at a rate different from that of groups 1 and 2 during weeks 7 and 8, were compared for each week and period of the trial, allowing for different order of administration of preparations, by means of analysis of variance. The mean daily cigarette consumptions for each period in study 1 (43 subjects) were: run in (weeks 1 and 2) 23.5; EIO (weeks 3-6 or 7-10) 24.4; dummy (weeks 7-10 or 3-6) 24.0; run out (weeks 11 and 12) 25.4 (36 subjects). Individual subjects' mean daily consumption varied between 12 and 42 per day in the run in period, individual extreme consumptions being 6 and 54. Twenty-three subjects' mean daily consumptions were higher in the EIO period than the dummy period; fifteen consumed less in the EIO period and five did not change. The two largest individual changes in daily consumption between periods on EIO and dummy were respectively a rise from 21 to 27 (average 21 on run in) and a fall from 32 to 25 (26 on run in).

The study finished just before a Christmas holiday when unusual social factors affect smoking habits. The study of the mature oats was therefore postponed. As the volunteers were no longer naïve (nor could any others from the same community be expected to be) dummies were not given during the run in periods. Instead, one-half took immature oats infusion over weeks 2 to 4 while the other took mature oats and in weeks 5 to 7 each group took the alternative. No significant differences between EIO and EMO were evident. Mean daily cigarette consumptions in study 2 (18 subjects) were run in 25.6, EIO 24.7 (14 subjects) and EMO 24.9. Four subjects stopped smoking after completing the second study because the 'budget' raised the cost of cigarettes. Regretfully we conclude that the views of our compatriot, Dr Samuel Johnson, on oats would not have been modified by our study, though by its lucky timing his views on excise might have been.

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### Marijuana metabolites measured by a radioimmune technique

THE recent surge of interest in the pharmacology of cannabinoids has created a need for a rapid assay for  $\Delta^9$ -tetrahydrocannabinol  $(\Delta^{0}$ -THC) and its metabolites in body fluids. A sensitive immune technique has proved elusive, and quantitation requires a multistep chromatographic and mass spectroscopic analysis<sup>1</sup>. Gas liquid chromatography with electron capture detection has also been used<sup>2,3</sup>. We describe here a simple rapid radioimmune assay for  $\Delta^9$ -THC utilising goat antiserum obtained as previously described4.

A standard antibody-binding curve was obtained as follows: 4.5 ng (5,000 c.p.m.) of <sup>3</sup>H-Δ<sup>8</sup>-THC (specific activity 465 mCimMol  $^{-1}, more than 98\,\%$  pure, New England Nuclear Corp.) was incubated for 2 h at 4° C with increasing dilutions of antiserum (0.3 ml). The latter were made up with phosphatebuffered saline (PBS), 0.1 M in phosphate, pH 7.0 in 0.1% human  $\gamma$  globulin. After this initial incubation, 1 ml of a cold

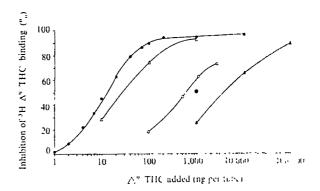


Fig. 1 Inhibition of <sup>3</sup>H-Δ<sup>9</sup>-THC binding to antibody increasing amounts of unlabelled THC metabolites. •, Δ°-THC; Δ, 11-hydroxy-Δ°-THC; Ο, 11-nor-9-carboxy-Δ°-THC; Δ, cannabidiol.

dextran charcoal suspension (0.25% dextran-2.5% activated charcoal in PBS), was added and incubation continued for 15 min. The tubes were then centrifuged at 3,000 r.p.m. for 15 min at 4° C and the supernatant (1 ml) was counted in a Nuclear Chicago liquid scintillation spectrometer (70% tritium counting efficiency) with Aquasol (New England Nuclear Corp.), as the scintillation fluid. Binding of <sup>3</sup>H-Δ<sup>8</sup>-THC by normal goat serum under the foregoing conditions was slightly above background. A maximum of 50% of marker was bound by a 1,60 early antiserum dilution. The latter serum dilution was used in the inhibition experiments described below.

To ascertain the specificity of the antiserum, various amounts of cold Δ9-THC, 11-hydroxy-Δ9-THC, 11-nor-9carboxy-Δ°-THC and cannabidiol were added to a 1/60 dilution of antiserum 30 min before addition of 4.5 ng <sup>3</sup>H-Δ<sup>6</sup>-THC. The sensitivity of the inhibition assay was 1.2 ng per tube at a 95% confidence limit. A sample inhibition experiment at 50% binding is plotted in Fig. 1 and percentage cross reactivities are tabulated in Table 1.

Table 1 Cross reactions of canna	binoids with THC antiserum
A <sup>p</sup> -THC 11-Hydroxy-THC 11-Nor-9-carboxy-THC Cannabidiol	% Cross reaction 100 47 2.6 0.5

\*At 50% inhibition

The association constant for the antiserum was determined graphically from a Scatchard plot. In the range of dilutions tested, crude antiserum bound THC at  $K_a = 4.0 \times 10^8$  M indicating a potential sensitivity of 300 pg per tube.

After a preliminary adsorption with charcoal5, samples of male plasma treated with known quantities of Δ9-THC (50-700 ng ml-1) were measured. Samples of 1 ml were shaken with heptane ethanol (100:1.5), the extracts were dried and the residue was dissolved in 0.5 ml of 50% ethanol. This solution (50 μl) was used in inhibition assays. Ideally the assays should have shown 5, 10, 20, 50 and 70 µg per tube, respectively. Their deviation from these points on the standard curve (Fig. 2) was small and within the limits of experimental error.

Plasma samples were obtained from chronic users of marijuana who were part of a controlled marijuana research project and had used no other drug for at least 10 d previously. Blood was drawn 15 min after they had smoked a marijuana cigarette (0.9 g) containing 2.1 % (18.9 mg)  $\Delta^{o}$ -THC. Plasma was separated by centrifugation and stored frozen at - 10° C. These samples (1-3 ml) were extracted with heptane-ethanol. Aliquots (0.25 ml) of reconstituted extract were used in the radioimmune assay system already described.

In each of six plasma samples  $\Delta^9$ -THC was detected and quantitated 30 min before and 15 min after THC use. Preintoxication levels were 60-100 ng ml<sup>-1</sup>. Postintoxication levels

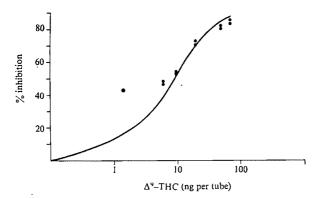


Fig. 2 Percentage inhibition of  ${}^3H-\Delta^8$ -THC-azoTHC binding by (—) various amounts of cold  $\Delta^9$ -THC added from a stock reagent vial, and (:) various amounts of cold  $\Delta^9$ -THC extracted from serum samples after treatment with  $\Delta^{0}$ -THC. Tests were done in duplicate.

were 200-250 ng ml<sup>-1</sup>. This represents 0.032% of total THC in the standardised cigarette. A significant portion of the remainder was presumed to have been exhaled. The amount of THC distributed in other body compartments is an important consideration but not of concern here.

Immunisation with Δ9-THC protein conjugates and testing of antibodies with plasma samples have been hampered by the relative insolubility and nonspecific binding of THC to serum protein. This binding can result in very high blanks and thus interfere with inhibition and assay interpretation. This was overcome in our early studies involving fluorescence by minimal fluorescence perturbation with nonspecific THC-protein binding and in recent experiments involving radioimmune methods through manipulation of the charcoal suspension.

Our antiserum was characterised by a low cross reactivity with closely related THC metabolites and other cannabinoids, the only exception being 11-hydroxy-Δ9-THC, now considered an insignificant contributor (paper presented by M. Wall at International Congress of Pharmaceutical Sciences, Sweden, 1973). The relative insolubility of 11-nor-9-carboxy- $\Delta^{0}$ -THC in the extraction solvent (heptane-ethanol) and its low cross reactivity with  $\Delta^9$ -THC makes its contribution negligible.

Although the sensitivity of our method is limited to 25-50 ng ml $^{-1}$  (4.5 ng of  $^{3}$ H- $\Delta^{8}$ -THC is required to provide 2,000 c.p.m. per sample tube), it can be used to estimate cannabinoids in chronic marijuana users. We are developing markers with much higher specific activities (100-200 Ci mmol<sup>-1</sup>) to enhance sensitivity into the pg ml<sup>-1</sup> range, for clearance studies and investigation of occasional abusers.

For body fluid determinations to be made several hours or more after exposure to marijuana, it would seem desirable to detect 11-nor-9-carboxy-THC6. Therefore we are preparing antibodies which are directed separately to the latter metabolite\*. Thus body fluid and tissue contents of each metabolite should more easily be correlated with psychoactive effects than was previously possible.

This work was supported in part by a grant from the National Institute of Mental Health Center for Studies of Narcotics and Drug Abuse.

\* Note added in proof: We now have a simple radioimmune assay specifically towards this important metabolite.

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### Cortical potentials evoked by finger displacement in man

THE neuropsychophysiology underlying the sense and change of limb position in animals and man is a centre of enquiry by many disciplines ranging from medicine and physiology to psychology and sociology1. Consistent changes in the electrocorticogram (ECoG) following change of position have, however, not yet been reported in man, although recordings of unit activity during neurosurgical operations have demonstrated neuronal activity in cortex<sup>2</sup> and subcortical structures3-5. Limited data from scalp recordings after passive movement have also been reported<sup>6,7</sup>.

Electrical changes, time-locked to movement of the index finger, have now been recorded from twelve subjects using cortical and scalp electrodes. The cortical recordings have been obtained from chronically implanted subdural gold wire electrodes in seven subjects selected for treatment by multifocal leukocoagulation8,9. Simultaneous cortical and scalp recordings were taken from these patients. Scalp recordings have been obtained from five normal subjects. The position of the cortical electrodes was determined by X-ray photography and by the reversal across the central fissure of the early negative component of the somatosensoryevoked responses after contralateral median nerve stimulation10-12. Precise localisation of the electrodes was not always possible and they have been grouped in three areas: (1) postcentral, immediately posterior, and (2) precentral, immediately anterior, to the central sulcus; and (3) prefrontal, close to the tip of the frontal lobe. All electrodes were in the right hemisphere about 5 cm lateral to the saggital plane. The scalp electrodes were placed 2 cm above the nasion to monitor eye movements, at the vertex, and over the preand postcentral cortical areas of both hemispheres.

Displacement of the left or right index finger by about 45° around the metacarpophalangeal joint was by a mechanical arrangement which permitted unobstructed return of the finger to the resting position. The displacement was monitored using a movement transducer. The differentiated and grossly amplified output of this transducer was used to trigger the PDP-12 computer used for multichannel data collection of the electrical brain activity for 1 s before and 1 s after the displacement. The electromyogram (EMG) from the arm flexors and extensors were also recorded. Time constant and upper frequency response of the electroencephalogram were 1.2 s and 700 Hz, respectively.

In all subjects, cortical and scalp recordings showed consistent responses after displacement. Figure 1 shows the responses recorded from three cortical and one scalp electrode to contralateral and ipsilateral displacement. To contralateral displacement the pre- and postcentral cortical areas showed an initial positivity with a delay of 34±6 ms to peak and  $42\pm4$  ms, respectively. The amplitudes ranged from 5 to 25 µV across subjects and were usually larger in postcentral areas. This positivity was followed by a negative wave with a peak latency of  $68 \pm 15$  ms for precentral and 97±20 ms for postcentral areas, its amplitude ranging from 30 to 50  $\mu$ V across subjects. The response continued for 400 ms with a number of positive and negative components. These late components, although consistent for an individual, were variable across subjects. In the prefrontal

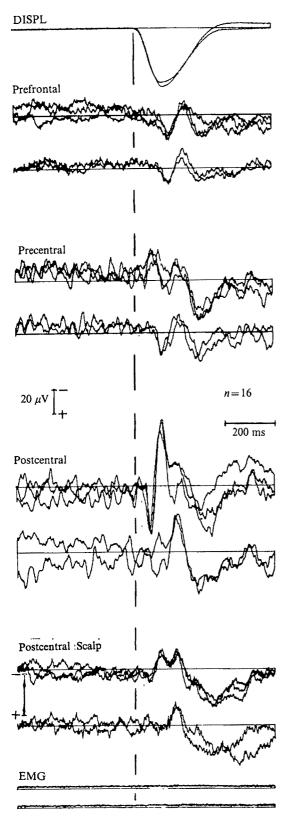


Fig. 1 Superimposed average cortical and scalp responses following externally paced left (upper trace of every pair) and right (lower trace) index finger displacement (DISPL). Interrupted vertical line shows the trigger point. Both cortical and scalp recordings from the right hemisphere with electrodes at prefrontal, precentral and postcentral cortices. The electromyogram (EMG) of the arm flexors does not change after displacement. Note the consistency in waveform for the same area under similar stimulus condition, the differences in waveform for different areas simultaneously registered and the different response of the same area, except prefrontal, to contralateral and ipsilateral displacement. Cortical electrodes referred to average of 60 electrodes in frontal lobe. Scalp electrodes referred to commoned electrodes on each mastoid process.

areas the responses to contralateral displacement started later than 100 ms and were similar to those evoked by ipsilateral movement. After ipsilateral finger movement, the pre- and postcentral areas gave different responses from those after contralateral displacement. The ipsilateral responses started with a positive deflection with a latency of 60 ms or more. Comparisons within subjects, of the total response to contralateral displacement from prefrontal, precentral and postcentral areas, showed different waveforms. Cross-correlations of responses recorded from electrodes within any one of these areas were high (about 0.85) but cross-correlations between responses from different areas were low (about 0.25).

Flexion or extension of the limb did not change the configuration or the spatial distribution of the displacementevoked potentials. This and the absence of EMG activity

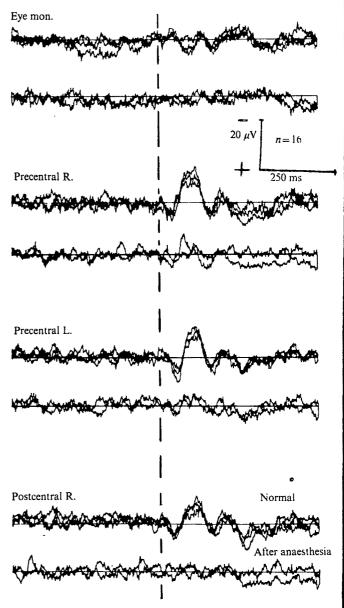


Fig. 2 Superimposed average, scalp-recorded, responses following externally paced left index finger displacement. Interrupted vertical line shows the trigger point. Eye mon., activity at an electrode 2 cm above the nasion for monitoring eye movements. Electrodes corresponding to precentral and postcentral cortical areas of the left or right hemisphere are marked L or R Upper trace of every pair, normal activity of the indicated location before, during and after displacement. Lower traces, activity of the same locations to similar displacement 45-55 min after anaesthesia of the hand with anoxia (AN). Note the loss of the responses after anaesthesia.

during the displacement indicate that muscle afferents contribute very little, if at all, to this response. To test this hypothesis anaesthesia of the joint has been imposed through ischaemic anoxia in normal volunteers, as previously described13. For 10 min the blood circulation in arm and hand was stopped by applying pressure with the cuff of a manometer just above the elbow joint. A second manometer encircled the wrist and was inflated and the pressure above the elbow relieved, thus permitting free blood circulation for the arm muscles. Scalp responses evoked between 45 and 55 min after anoxia are compared with the pre-anoxic responses in Fig. 2. The response is greatly reduced although the muscles of the arm and their sense organs are in normal conditions. We conclude that muscle afferents do not contribute to the displacement-evoked responses which most probably reflect activation of joint afferents. Activation of these afferents evokes different electrical changes in prefrontal, precentral and postcentral cortical areas. This differential representation in the cortical level of the input activated by displacement, as well as the timing of the various components of the response, offer an objective index in understanding the brain mechanisms of position sense in normal man and in patients.

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### Visualisation of micropore structure in human dental enamel

Dental enamel has an internal void volume of 0.2-5%1-3. The direct observation of pores in enamel, however, has been hindered by inadequate specimen preparation techniques. The harsh chemical treatment necessary to partially demineralise enamel prior to embedding and sectioning, and the chatter and shattering effects which result from cutting hard tissues. create artefactual spaces in enamel which make it virtually impossible to identify and define any natural spaces present. Attempts to cut enamel which has not been demineralised produce even greater shatter and destruction of the natural structure.



Transmission electron micrograph of longitudinal section of ion beam thinned human dental enamel showing the long lath structure of apatite crystals with electron lucent channels or pores between them. Ion beam thinning may remove organic material faster than the mineral phase but because it is a mild process in which the incoming ions knock out individual surface atoms it does not result in any detectable gross morphological artefactual damage as produced by other ultra sectioning techniques. Human teeth extracted from young healthy adults 20 to 30 years of age were obtained from the Royal Dental Hospital of Melbourne and fixed in 10% v/v isotonic buffered formal saline for 24 h at room temperature. After washing in water and air drying, the teeth were embedded in Servic polyester resin (obtained from R. F. Service, 272 Brunswick Street, Fitzroy, Melbourne) and cured for 24 h (×180,000).

We have observed with the electron microscope a micropore structure in human dental enamel using an improved technique in which specimens are prepared by alumina jet dimpling followed by ion beam thinning4. This technique enables the preselection of a defined area for preferential thinning, making it suitable for electron transmission.

Longitudinal serial sections (300 µm thick) of resin-embedded teeth were prepared by the method of Malcolm<sup>5</sup>. After lapping by hand to approximately 100 µm thickness and after polishing both sides, the sections were mounted on glass slides using an alcohol-soluble medium, such as canada balsam. Using a light microscope, areas of enamel were selected in each specimen (a) in the vicinity of the dentino-enamel junction in the cusp region and (b) midway between the surface and the dentino-enamel junction in the central region between cusp tip and the cervix. These areas were then enclosed by cementing a slotted grid or ring to the specimen with a rapid-setting alcohol-insoluble cement. The grid or ring also provides support for the specimen. A dimple (diameter < 1 mm) was made on the area selected by accurate positioning under a jet of fine alumina directed from a fine silicon carbide nozzle. Rate of dimpling was controlled by the nitrogen gas pressure operating the jet; the use of different size jets, combined with varying

specimen-jet distances, enabled determination of the area thinned. A specimen thickness of approximately 30 µm in the dimpled area was found suitable for ion beam thinning; this was measured optically or using a dial gauge. After dimpling, the disk was separated from the glass base by immersion in alcohol and then trimmed with a sharp knife. The disk, mounted in a stainless steel holder, was tilted to approximately 15° to the plasma beam in an ion beam thinning unit. Perforation of the dimpled area (monitored microscopically) was the criteria determining suitability for electron microscopy. The exact identification in the electron microscope (JEM 200 kV) of specific features noted optically was achieved by careful photographic comparison before and after thinning. Electron diffraction was used to determine the precise orientation of enamel crystals in any one field. The transmission electron microscope observations showed that human dental enamel prepared as above had no detectable gross morphological artefactual damage found with other ultra-sectioning techniques.

In longitudinal section, enamel crystals appeared to be long lath or ribbon-like structures extending across the field without interruption, except at prism boundaries. These crystals (approximately 30-90 nm wide) appeared to be of indeterminate length with none of the cross fractures usually observed in electron micrographs of enamel crystals. The most striking feature in all areas of the enamel examined, however, was the presence of electron-lucent channels running between, and parallel to, the crystals (Figs 1 and 2). These channels or pores (1-10 nm wide; average width about 5 nm), like the crystals, appeared to be of indeterminate length in longitudinal sections; they may run for long distances through the enamel rods and



Fig. 2 Transmission electron micrograph of transverse section of ion beam thinned human dental enamel showing flattened hexagonal apatite crystals with irregularly shaped holes separating the individual crytsals. ( $\times$ 80,000)

only terminate at a rod boundary or free surface. They were best observed in slightly out of focus micrographs because of enhanced contrast due to Fresnel fringes. But the width measurements were made from in-focus micrographs. In cross section, these channels appeared as irregular shaped holes of similar diameter to that measured for the longitudinally sectioned channels. It was impossible to tell from the electron micrographs whether the channels had a material structure of their own or were merely spaces created by the packing arrangements of the crystals. The electron micrographs gave the impression that these channels were empty spaces, however, they may have contained soft material, for example organic matter, preferentially removed by the ion beam thinning.

An approximate estimate of the relative percentage volume of these pores was made by examining over 200 electron micrographs of numerous fields. The pore area and total area were calculated using a ruled grid on a transparent overlay and then multiplied by average pore diameter and crystal thickness, respectively, obtained from electron micrographs. Determination of the percentage pore volume relative to total enamel volume for a section of enamel one crystal thick gives a range from 1 to 5%, using the widest range of pore diameters.

Although this calculation is approximate, the close agreement with established figures1 is strong evidence that the pores observed here do represent the spaces in enamel, detected by indirect means.

Experimentation<sup>6-8</sup> has shown that human teeth are permeable to ions and some molecules which can pass from the pulp to the enamel surface or vice versa. The presence in the enamel of a system of pores or channels (average diameter 5 nm), which would permit the passage of ions and small molecules, would explain the permeability of this material. It must also have considerable significance for its physicochemical behaviour, particularly with regard to dissolution, remineralisation and carious destruction. These aspects are being investigated currently by a detailed quantitative analysis of the distribution and size range of these micropores in both normal and pathological enamel from cusp tip to cervix and from surface to dentino-enamel junction.

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### Oral contraceptive for men

We have induced reversible infertility, with little effect on libido, in five healthy young men by giving them tablets containing methyltestosterone and ethynyloestradiol. There were no undesirable side effects and we feel that this treatment may offer a practical approach to male oral contraception.

The acceptability of oral contraceptives for women has led many people to investigate the possibility of producing similar products for use by men<sup>1</sup>. The major mechanism of action of synthetic steroid hormones in oral contraceptives is inhibition of ovulation, due to suppression of the mid-cycle surge of gonadotrophins released by the pituitary<sup>2</sup>. Oral contraceptives inhibit spermatogenesis by a similar mechanism, but so far there has been concomitant loss of libido and potency<sup>3</sup>. Clinical results<sup>4-6</sup>, however, have shown that this can be avoided if supplementary androgens are given in combination with an anti-gonadotrophic steroid such as danazol4, norgestrienone5, or ethylnorgestrienone6. But clinical testing of such combinations on a large scale cannot begin without extensive toxicological testing with animals<sup>7</sup>. We have therefore reconsidered existing oral hormone products which have been approved for sale in many countries. One group of these products<sup>8</sup> contains combinations of an androgen (usually methyltestosterone: 17α-methyl-17β-hydroxy-4-androsten-3one) with an oestrogen (usually ethynyloestradiol: 17aethynyl-1,3,5(10)-estratriene-3,17 $\beta$ -diol). They are sold for treatment of, for example, osteoporosis in men and the symptoms of 'male menopause'.

Initial studies were conducted on two men with osteoporosis who had been receiving products of this type for several months. Both proved to be aspermic, but reported no undesirable side effects, apart from a transitory nausea during the first few days. Concentrations of bilirubin, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase and albumin in these men were within normal limits. With this background, we decided to test an oral androgen-oestrogen product as a contraceptive in healthy male volunteers.

The concentration of testosterone in the plasma of healthy men averages 4-12 ng ml<sup>-1</sup>, with a daily production of 5-10 mg9. Methyltestosterone has a longer biological halflife than testosterone, but a 10 mg tablet is necessary to maintain concentrations of 4-15 ng ml<sup>-1</sup> in the plasma for up to 8 h (ref. 10). We therefore used tablets containing 10 mg methyltestosterone plus 20 µg ethynyloestradiol. This combination was taken twice daily at 0800h and 1800h with meals. A recent study<sup>11</sup> suggested that this dose of oestrogen is sufficient to suppress pituitary secretion of follicle-stimulating hormone (FSH) in normal adult men. To evaluate possible side effects we compared the active hormone preparation with a placebo in the same patients. As supplies of matching placebo tablets were unavailable, the active tablets were crushed and repacked in gelatin capsules. Identical placebo capsules were packed with the same quantity of lactose. Five healthy male subjects (23-38 yr old) received daily placebo capsules for 3 weeks before taking the hormone product, but were unaware of this. The wives of the subjects all used combined-type oral contraceptives and continued to do so until advised to the contrary.

Blood and semen were collected at the start of the study and after 3, 6, 12, 18 and 24 weeks. Routine biochemical analysis of blood plasma indicated no hepatotoxicity. All semen specimens were normal at the beginning and end of the period when placebo was given, but sperm numbers and motility had decreased significantly by week 12 (that is day 63 of hormone treatment). At this time, four of the subjects were aspermic and remained so for the duration of treatment. The other subject produced an aspermic semen specimen during week 18. Semen specimens were collected at approximately monthly intervals after treatment was stopped. There was no increase in sperm in any of the men for about 15 weeks after the cessation of hormone treatment, but all then began to show a gradual restoration. Normal sperm numbers and motility were found after 35-40 weeks without treatment.

We conclude that the androgen oestrogen combination inhibited spermatogenesis, but that the effect was reversible when treatment stopped. Two subjects reported decreased libido during the initial 8 weeks (which included the placebo

period), but normal libido for the remainder of the study. Another man reported increased libido during the second half of the treatment period, while he and another subject experienced a reduction in libido after treatment was stopped.

Three subjects reported occasional mild nausea, sometimes while they were taking the placebo. No nausea was reported after week 18. There were no changes in skin, hair, breasts or micturation. When the wives of the volunteers stopped taking their oral contraceptives between weeks 18 and 34, no pregnancies occurred, as would have been expected with aspermic men.

Thus we believe that hormone preparations already being marketed for other purposes could be used as oral contraceptives for men. We recommend that these preparations be investigated further to establish the most suitable doses and administration regimen. It seems possible that similar effectiveness could be obtained with discontinuous treatment.

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### LSD as an agonist of dopamine receptors in the striatum

THE mechanism of the hallucinogenic action of (+)lysergic acid diethylamide (LSD) is still obscure. Its molecular structure (containing both an indole and a phenylethylamine moiety) suggests the possibility of an interaction with brain monoamines. The earliest hypothesis postulated an antagonistic action of LSD at 5-hydroxytryptamine (5-HT) receptors in the brain1,2. More recent data rather tend to support the view that the hallucinogen mimics at least some of the effects of endogenous cerebral 5-HT3-5 Inhibition of the release of 5-HT is another suggested mechanism of action<sup>6</sup>. An interaction of LSD with dopaminergic transmission has so far not been demonstrated, although the marked attenuation of LSD-induced symptoms by chlorpromazine and other antipsychotic drugs7-8 may suggest this. Using a modification of the rotational model proposed by Ungerstedt10 we found that LSD acted as a potent agonist at dopamine receptors in the striatum.

A unilateral lesion of the nigro-striatal dopamine system of rats lowers the dopamine content in the striatum on that side. The resulting imbalance between the dopaminergic activities in the right and left striatum leads to postural and locomotor asymmetry both spontaneously and, even more markedly, in response to certain agents. Thus, drugs which enhance the release of dopamine from nerve terminals in the striatum of the intact side, for example, amphetamine, induce a rotation towards the side with the

lesion (ipsilateral circling) possibly by removing an inhibitory influence of the striatum upon the motor activity of the side of the body contralateral to the lesion. Conversely, agents like apomorphine, which stimulate dopamine receptors directly, induce rotation towards the intact side (contralateral circling), probably due to exaggerated responsiveness of the denervated striatum to these agents<sup>10</sup>.

Unilateral lesions of the nigro-striatal dopamine system were produced in male SPF rats of the stock Füllinsdorf albino by the injection into the right medial forebrain bundle (MFB) of either 3.5 µg 6-hydroxydopamine (6-OHdopamine) in 4  $\mu$ l Ringer's solution containing 0.02% ascorbic acid, or 10 µg 5,6-dihydroxytryptamine (5,6-HT) in 10  $\mu$ l of same. Using these two neurotoxic agents we obtained two groups of animals similarly depleted of telediencephalic dopamine on the side of the injection but differing in the levels of the other monoamine transmitters: 6-OH-dopamine lowered dopamine and noradrenaline levels (L.P., M.P., and W.H., in preparation), but did not alter the 5-HT content; the injection of 5,6-HT depleted dopamine and 5-HT but did not affect noradrenaline 11,12. For the measurement of drug-induced behaviour, rats were gently placed in a plexiglas box (35×20×15 cm) immediately following the intraperitoneal (i.p.) injection of the compounds. Handling alone induces in most lesioned animals a 'paradoxical' contralateral rotation10, however, which never lasts more than 3 min. The circlings during the first 5 min were therefore not recorded. The intensity of the rotational behaviour was expressed as the total number of full turns counted during 2 min at intervals of 5 min. Beginning on the 10th postoperative day, all rats were tested for their rotational responses to (+)-methamphetamine and apomorphine before LSD was given. A drug-free interval of one week was allowed between tests. As expected, the responses to (+)-methamphetamine (1-5 mg kg<sup>-1</sup> i.p.) and apomorphine (0.05-3 mg kg<sup>-1</sup> i.p.) were ipsilateral and contralateral circling, respectively. Animals treated with 6-OH-dopamine and with 5,6-HT did not differ in their responses. Ten shamoperated rats (local injection of solvent into the MFB) did not rotate in response to apomorphine and amphetamine.

LSD (tartrate) (100  $\mu$ g kg<sup>-1</sup> i.p., but not 50  $\mu$ g kg<sup>-1</sup> i.p.) induced contralateral turning, which began after 5 min and reached a peak after 15 min (Fig. 1). Doses higher than 100  $\mu$ g kg<sup>-1</sup> did not increase further the number of turns per minute but the peak of rotational behaviour was pro-

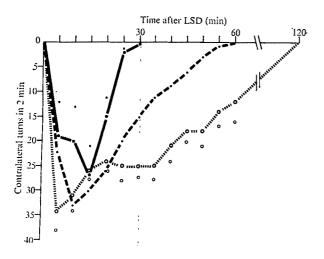


Fig. 1 Contralateral rotation (towards the unoperated side) induced by three doses of LSD injected i.p. in rats with lesions of the right medial forebrain bundle produced by the local injection of 5,6-dihydroxytryptamine. The intensity of the rotational behaviour is indicated by the number of turns in 2 min (means of ten animals per dose) s.e.m. Indicated by small points or circles. LSD doses: \_\_\_\_\_\_, 100  $\mu$ g kg<sup>-1</sup>; \_\_\_\_\_\_ 200  $\mu$ g kg<sup>-1</sup>; \_\_\_\_\_\_ 1,500  $\mu$ g kg<sup>-1</sup>.

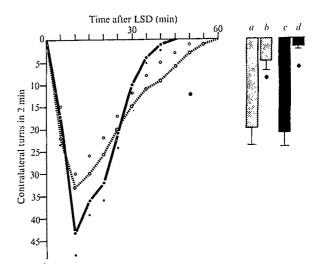


Fig. 2 Comparison of the rotational response elicited by LSD (200 μg kg<sup>-1</sup> i.p.) in rats with lesions of the right medial forebrain bundle produced by 6-hydroxydopamine (n=10) and by 5,6-dihydroxytryptamine (n=10). The right-hand side shows the effect of haloperidol (1 mg kg<sup>-1</sup>) injected 1/min after LSD. a and c, Turning activity in the two group of rats, 25 min after LSD alone; b and d, turning activity under the effect of haloperidol. Means ± s.e.m. (n=10). T difference between LSD alone and LSD+haloperidol statistically significant (P<0.01, Student's t test, two-tails Solid line and bars, 6-hydroxydopamine; dotted line stippled bars 5,6-hydroxytryptamine.

longed in a dose dependent way. The time-course o tion after LSD (100 µg kg) paralleled the temporal c of the levels of LSD in the brain13. Repeated inject LSD (200 µg kg<sup>-1</sup> i.p.) on 5 consecutive days, a s reported to induce tolerance to the hallucinogen in behaviour studies14, did not reduce the rotational re There was no significant difference between rats with 6-OH-dopamine and those lesioned with 5 the intensity and duration of contralateral circli ponse to LSD (200 µg kg<sup>-1</sup>) (Fig. 2). In both animals the injection of haloperidol (1 mg kg-1 i after the administration of LSD (200 µg kg<sup>-1</sup>) s reduced the circling behaviour (Fig. 2). Pretre before LSD, with  $\alpha$ -methyl-L-tyrosine ( $\alpha$ -MT) (' i.p.), an inhibitor of the synthesis of catecho not alter the rotational response in spite ( marked sedation. (+)-2-Bromo-lysergic acid bitartrate (BOL-148), up to 20 mg kg<sup>-1</sup> i.p., induce circling in either group of lesioned an the doses used had no detectable effect in rats.

Thus, in rats with the nigro-striatal do lesioned unilaterally by injection into the different neurotoxic agents (6-OH-dopamir LSD induced circling towards the intact side apomorphine. It seems reasonable to cor is a potent agonist at central dopamine rece depletion of noradrenaline and 5-HT in reduction of dopamine and the inhibition synthesis by a-MT had no influence on therefore, the mechanism involved in the to LSD seems to be purely dopaminergi-Haloperidol, a dopamine receptor antal effect of LSD as well as that of ap L-dopa). Further support for a dopamin action of LSD is provided firstly by its turnover of dopamine in the rat bi personal communication), secondly b weaker than that of DA, to activate cyclase in a homogenate of rat stria personal communication), and finall

cyclic adenosine 3',5'-monophosphate in rat cerebrum after its i.p. administration15. BOL-148, an analogue of LSD believed to have very weak, if any, hallucinogenic properties<sup>61</sup>, did not induce circling behaviour. In preliminary experiments, methysergide, another non-hallucinogenic lysergic acid derivative, and N,N-dimethyltryptamine, an idolealkylamine hallucinogen, were also inactive as dopamine receptor agonists. On the other hand, such an action has recently been described for some ergot alkaloids and derivates (ergocornine, 2-bromo- $\alpha$ -ergocryptine, metrine)17,18

The activation of striatal dopamine receptors is obviously not the only important central effect of LSD, since its overall behavioural effects differ considerably from those of agents believed to be fairly pure agonists of dopamine receptors. The possible importance of the dopaminergic component of the action of LSD for its hallucinogenic effect has yet to be demonstrated. It will be interesting to see whether LSD also activates dopamine receptors in brain regions other than the striatum, for example, in the limbic subsortical and cortical18,18 structures, in the hypothalamus, in the medullary chemical trigger zone, and in the retina<sup>22,23</sup>.

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### LSD as an agonist and antagonist at central dopamine receptors

THE mechanisms involved in the psychotomimetic actions of D-lysergic acid diethylamide (D-LSD) and other hallucinogenic agents have not been defined. Neurophysiological and behavioural studies indicate that p-LSD may interact with serotonin and catecholamine receptors in the central nervous system. Thus, this drug seems to stimulate certain central serotonergic pathways<sup>1,2</sup>, while inhibiting the activation of other pathways by serotonin3,4.

D-LSD has also been reported to block the action of noradrenaline on central neurones<sup>5</sup> and to produce stereotypy in rats<sup>6</sup>, a condition which is associated with dopaminergic hyperactivity. Moreover, several recent reports have indicated that a number of ergot derivatives are capable of exerting dopamine-like actions on central neurones in the rat8-10. Recent investigations from this laboratory suggest that adenyl cyclase systems may be involved in the central actions of D-LSD (ref. 11 and K.v H., S.R., and D.F., unpublished). Thus, D-LSD is capable of stimulating adenyl cyclase activity, as well as antagonising the stimulation of this enzyme by serotonin, in cell-free preparations from the rat colliculus. Here we report evidence that D-LSD may act both as an agonist and an antagonist at dopamine receptors in the brain.

The methods employed for the preparation of brain subcellular fractions and measurement of adenyl cyclase activity have been described in detail elsewhere12,13. Brains were obtained from young, adult male rats of an inbred Sprague-Dawley strain. These rats were approximately 6 weeks old and weighed about 225 g. Particulate fractions were prepared from cerebral cortex or corpus striatum.

In relatively low concentration (10 µM), D-LSD, completely blocked the stimulation of cerebral adenyl cyclase activity by a combination of 10  $\mu M$  noradrenaline and 10  $\mu M$  dopamine (Table 1). At these concentrations, the catecholamines produced additive effects on enzyme activity12. Another serotonin antagonist, 2-bromo-D-lysergic acid diethylamide (BOL) also inhibited this response to the catecholamines. Comparable results were obtained when these serotonin antagonists at 100 µM concentrations were tested against equimolar amounts of either noradrenaline or dopamine. D-LSD also inhibited

Table 1 Influence of lysergic acid derivatives on activation of adenyl cyclase by catecholamines in particulate preparations from cerebral cortex of adult rats

The state of the s			
	Cyclic AMP form	ned	
	•	Increase with	
Lysergic acid	Basal	catecholamines	
derivative	(nmolmg <sup>-1</sup> protein h <sup>-1</sup> )	(nmolmg-1 protein h-	¹)(%)
None	$8.46 \pm 0.18$	$6.39 \pm 0.22$	76
D-LSD, 10 μM	$9.46 \pm 0.26$	$0.31 \pm 0.29*$	3
L-LSD, 80 μM	$8.02 \pm 0.15$	$5.63 \pm 0.49$	70
BOL, 10 uM	$7.60 \pm 0.28$	$0.91 \pm 0.41*$	12

The particulate preparations were crude mitochondrial fractions obtained by centrifuging the 1,000g supernatant fluids from cerebral homogenates at 10,000g for 20 min. Adenyl cyclase activity was determined from the conversion of <sup>14</sup>C-ATP to cyclic AMP by a procedure<sup>12</sup> which was based on the method of Krishna et al.<sup>14</sup>. The incubation medium (0.1 ml) contained the following components in the final concentrations indicated: 3.6 mM (0.5 μCi) 8-14C-ATP, 5.0 mM MgCl<sub>2</sub>, 1.0 mM cyclic AMP, 40 mM Tris-HCl (pH 7.3 at 37° C), 10 mM phosphoenolpyruvate, 4  $\mu$ g pyruvate kinase, 15 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (added with pyruvate kinase), 20 mM caffeine, 0.1 mg bovine serum albumin, 0.2 mM EGTA, 5  $\mu$ g phosphatidylserine and 50-100 µg brain protein. Protein was measured by the method of Lowry et al. 15. L-Noradrenaline (10 µM) and dopamine (10 µM) were added together as the hydrochlorides, D-LSD and L-LSD as the free amines and BOL as the bitartrate. Incubation was conducted in air for 5 min. Values for cyclic AMP formed are averages ±s.e. for triplicate samples in a representative experiment. Where indicated, basal values shown were obtained in the presence of the lysergic acid

derivative. \*P < 0.001 for the difference between this value and the value obtained with only the catecholamines added. .

Table 2 Influence of blocking agents on activation of adenyl cyclase by dopamine and D-LSD in particulate preparations from corpus striatum of adult rats

	Basal	Cyclic AMP fo Increase with dopa	mine	Increase with D-	
Agent	(nmol mg <sup>-1</sup>	(nmol mg <sup>-1</sup>	(%)	(nmol mg <sup>-1</sup>	(%)
E-manimant 1	protein h <sup>-1</sup> )	protein h <sup>-1</sup> )		protein h <sup>-1</sup> )	
Experiment 1					•
None	$18.43 \pm 0.67$	$11.46 \pm 0.76$	62	$5.65 \pm 0.73$	31
D-LSD (10 μM)	$24.08 \pm 0.27$	$1.12 \pm 0.42*$	5	•	
Chlorpromazine (10 µM)	$17.34 \pm 0.31$	$0.49 \pm 0.76 \dagger$	3	$1.40 \pm 0.42 \dagger$	8
Haloperidol (10 µM)	$16.93 \pm 0.32$	$0.73 \pm 0.44 *$	4	$1.40\pm0.33\dagger$	8
Experiment 2		•			
None	$15.57 \pm 0.42$	$9.54 \pm 0.69$	61	$4.70 \pm 0.45$	30
MLD (100 μM)	$15.03 \pm 0.26$	$0.20\pm0.43*$	1	$-0.10\pm0.45\dagger$	-1
BOL (100 μM)	$15.13\pm0.48$	$-1.70\pm0.52*$	11	$-1.14\pm0.51\dagger$	-8
Propranolol (100 µM)	$16.03\pm0.29$	$8.13 \pm 0.51$	51	$3.90\pm0.57$	24
Cyproheptadine (100 µM)	$13.56 \pm 0.12$	$0.58 \pm 0.37*$	4	$1.31\pm0.31\dagger$	10

The particulate preparations were obtained by centrifuging the homogenates at 10,000g for 20 min. See Table 1 for other explanations.

\*P<0.001 for the difference between this value and the value obtained with only 10 μM dopamine or 10 µM D-LSD added.  $\dagger P < 0.01$ .

the adenyl cyclase response to noradrenaline or dopamine in particulate preparations from the rat hippocampus, which resembles cerebral cortex in the responses of adenyl cyclase to the catecholamines<sup>13</sup>. Palmer and Burks<sup>16</sup> earlier reported that D-LSD and BOL block the noradrenaline-induced increase in cyclic AMP levels in slices of rat brain.

By itself, D-LSD tended to increase adenyl cyclase activity in the cerebral and hippocampal preparations. This effect was quite small however, (about 15%) and was not always significant. A survey of various brain regions of the rat revealed that cellfree fractions from the corpus striatum contained adenyl cyclase which was reproducibly activated by low concentrations of D-LSD. Significant stimulation could be obtained with concentrations of D-LSD as low as 0.1 µM. Adenyl cyclase activity in comparable fractions from other regions of rat brain (cerebellum, hypothalamus and brainstem) was completely refractory to the stimulatory action of this drug.

Adenyl cyclase activity in particulate preparations from striatal tissue of the adult rat was increased about 30% by  $10\;\mu\text{M}$  D-LSD (Table 2). This was about one-half the activation produced by an equimolar concentration of dopamine. The sensitivity of adenyl cyclase to dopamine in cell-free fractions from rat corpus striatum has been demonstrated earlier<sup>13,17,18</sup>. This region of rat brain is unusually rich in dopaminergic neurones19. The response to 10 µM dopamine in our investigations was completely blocked by the addition of 10 μM D-LSD. In addition, the responses of adenyl cyclase to either 10 μM dopamine or 10 μM D-LSD were almost completely blocked by equimolar concentrations of the antipsychotic dopamine-blocking agents, haloperidol and chlorpromazine. The serotonin antagonists, BOL, 1-methyl-D-lysergic acid diethylamide (MLD) and cyproheptadine were also capable, however, of inhibiting these responses (Table 2, experiment 2). The β-adrenergic blocking agent, propranolol, which markedly inhibits the response of adenyl cyclase to noradrenaline in rat brain cell-free preparations<sup>12</sup>, did not block the stimulation of striatal adenyl cyclase by dopamine or D-LSD. None of these blocking agents by itself, with the exception of p-LSD, increased adenyl cyclase activity in the striatal preparations.

Our findings, combined with earlier observations from this laboratory11, indicate that D-LSD is capable of blocking the interaction of noradrenaline, dopamine and serotonin with their respective receptors in various regions of rat brain. This drug also seems to activate central serotonin and dopamine receptors for adenyl cyclase. Interaction of D-LSD with dopamine receptors was blocked by dopamine-blocking agents (for example, haloperidol) or serotonin antagonists (for example, cyproheptadine), but not by the noradrenaline antagonist, propranolol. These investigations suggest that the hallucinogenic actions of D-LSD may be due to complex agonist and antagonist actions of this drug at central receptors for the several neurotransmitter monoamines.

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### The activity of membrane bound enzymes in muscular dystrophic chicks

Tissues from patients with Duchenne muscular dystr/ (DMD) as well as animals with similar inherited2 or mentally produced3 disorders have been shown to

Table 1 Adenyl cyclase in dystrophic chick muscle sarcolemma (pmol cyclic AMP per mg protein per 5 min)									
Activity		embryos			lays			reeks	_
Basal GTP	Normal 29.8±2.3 37.8±0.4	Dystrophic 5.7±1.3 17.6±2.5	<i>P</i> < 0.01 < 0.01	Normal $28.0\pm2.7$ $32.3\pm0.9$	Dystrophic $29.5\pm0.1$ $38.9\pm3.6$	P NS <0.05	Normal 20.1±0.5 28.8±0.7	Dystrophic 58.7±7.8 102.8±13.6	<i>P</i> <0.01 <0.01

Muscle sarcolemma was prepared from the pectoralis of pooled muscular dystrophic chicks and age-matched white leghorn (No. 26 from Arbor Acres) control chicks by the method of Severson et al. 15. Adenyl cyclase was assayed by measuring the conversion of  $\alpha$ -32P-ATP into 3',5'-cyclic-AMP16. Membrane protein (100-150 µg per assay tube) were incubated for 5 min at 37° C at pH 8.3. Protein was determined by the method of Lowry et al. 12 using bovine serum albumin as standard. Each estimate represents the mean  $\pm$  s.d. of three determinations. Statistical significance of the differences between means was determined using Student's t test.

significant changes in the amount and composition of their membrane lipids. Most pronounced are the increases in cholesterol and sphingomyelin and the decrease in phosphatidyl choline levels. Functional anomalies observed in this disease, such as the impairment in sarcoplasmic reticulum calcium uptake<sup>4</sup> or the changes in the ATPase activity of the sarcolemma<sup>5</sup>, can be related to the lipid disorder.

Both the anomaly in lipid composition and related functional impairments have been observed in tissues other than muscle. For example, erythrocytes from patients with DMD were shown to differ from controls in their lipid composition<sup>1</sup>, in their surface properties as characterised by scanning electron microscope<sup>6</sup> and in membrane ATPase activity<sup>7</sup>. Similarly, in mice with muscular dystrophy the erythrocyte acetylcholine esterase activity was found to be half that of normal controls8. Such observations led to the hypothesis that the genetic basis of Duchenne muscular dystrophy is a defect in lipid metabolism<sup>1,2,9</sup> which may involve the plasma membrane<sup>4,10,11</sup>. Lipids are instrumental not only in the control of membrane selectivity and diffusion across the membrane12 but also in the determination of the activity of membrane bound enzymes and their modulation by hormones and other agents<sup>13,14</sup>. One would therefore expect that if muscular dystrophy is due to a lipid related plasma membrane disorder the activity of membrane associated enzymes and their susceptibility to stimulation should be affected. Involvement of multiple organs would support the hypothesis that the membrane defect is linked to the genetic cause of the disease.

In this study we have investigated adenyl cyclase and ATPase activity associated with the plasma membrane of muscle, liver and erythrocytes from dystrophic chicks and the appropriate controls, under conditions of enzyme stimulation and interference with membrane cholesterol. The adenyl cyclase activity of the muscle sarcolemma from dystrophic chicks differed significantly from that of age-matched normal chicks in several

respects (Table 1). Whereas in the normal chick the specific activity of the enzyme did not vary considerably with age, in the dystrophic it increased progressively to reach, at six weeks, twice the control level. The enhanced GTP (10<sup>-5</sup> M) stimulation of the enzyme in the dystrophic membrane (Table 1) suggests that the differences are due to the expression of enzyme activity rather than to the amount of enzyme present.

Enzyme activity is determined by the microenvironment which controls the conformation of the enzyme, its coupling to the stimulus-receptor site and the substrate accessibility. It has been shown recently that this microenvironment can be affected by amphotericin B, which interacts with cholesterol<sup>14</sup>. Addition of amphotericin B to the sarcolemma depressed the basal enzyme activity of the normal muscle more than that of the dystrophic one. Also, the ratio of the GTP-stimulated to the basal enzyme activity increased after amphotericin B treatment in the normal membrane but not in the dystrophic one (Table 2).

Significant differences were also observed in the adenyl cyclase of liver plasma membrane. As in the muscle of 6-week-old chicks, the basal enzyme activity was elevated in the dystrophic chicks. The percentage stimulation by 10<sup>-5</sup> M glucagon increased after amphotericin B treatment in dystrophic liver but decreased substantially in the normal liver membrane (Table 2). This reiterated the role of the lipid environment in the mediation of the hormone stimulation. Differences between liver and muscle membrane may be due either to tissue specificity or to the mode of action of the different stimulating agents.

In red blood cell membrane, the basal adenyl cyclase activity was lower in dystrophic chicks and showed substantially less stimulation by adrenaline. Digitonin treatment, which extracts membrane cholesterol, reduced the enzyme activity in the normal membrane and totally abolished it in the dystrophic membrane (Table 2).

Table 2 The effect of amphotericin B or digitonin on the plasma membrane adenyl cyclase of normal and dystrophic chicks (pmol cyclic AMP per mg protein per 5 min)

			-			
	C	ne	Treated membrane			
Activity	Basal	Stimulated	Stimulated/Basal	Basal	Stimulated	Stimulated/Basal
Tissue Muscle						
Normal Dystrophic	28.0±2.7 29.5±0.1 NS	32.3±0.9 38.9±3.6 P<0.05	$1.15 \pm 0.09$ $1.32 \pm 0.07$	$17.7 \pm 0.6$ $24.5 \pm 2.5$ $P < 0.01$	$24.0\pm0.3$ $30.0\pm0.5$ P<0.01	$1.34\pm0.03 \\ 1.22\pm0.08$
Liver	2.2	1 10.00		2 10102		
Normal Dystrophic	$111.9 \pm 2.2$ $136.9 \pm 9.6$ $P < 0.02$	$200.7\pm5.6$ $215.9\pm7.8$ $P < 0.05$	1.79±0.02 1.58±0.05	100.3±1.3 98.1±3.0 NS	$128.6 \pm 5.4$ $167.8 \pm 2.6$ $P < 0.01$	$1.28 \pm 0.03$ $1.71 \pm 0.02$
Erythrocytes						
Normal Dystrophic	$3.50\pm0.17$ $1.49\pm0.02$ $P<0.01$	6.13±0.65 1.87±0.30 <i>P</i> < <b>,</b> 0.01	1.75±0.07 1.20±0.12	$_{0.80\pm0.06}^{+0.06}$	$0.51 \pm 0.05 \\ 0$	0.90±0.19 —

Muscle sarcolemma was prepared by the method of Severson et al.<sup>15</sup>. Liver plasma membrane was prepared following the procedure of Neville <sup>18</sup> up to step 11 (partially purified membranes). Chick erythrocyte membranes were prepared by using a modification of the procedure of Oye and Sutherland<sup>19</sup>. Adenyl cyclase activity in muscle was assayed as described in Table 1. Liver plasma membrane protein (50–100 μg per assay tube) was incubated for 5 min at 37° C at pH 7.6. Erythrocyte membrane protein (3Q0–350 μg per assay tube) was used under the same conditions. Muscle adenyl cyclase was stimulated with 10-5 M GTP, liver with 10-5 M glucagon and erythrocyte membrane with 10-5 M adrenaline. Muscle and liver plasma membranes were treated with amphotericin B (Squibb intravenous preparation) to yield a concentration of 0.1 mM. Erythrocyte membrane was treated with 1 mg ml-1 digitonin as described by Pohl et al.<sup>13</sup>. Results represent the mean ± s.d.m. of three determinations, Statistical significance of the differences between means was determined using Student's t test. s.d. of the ratios, R, was computed according to σ<sub>R</sub>=[(σ²<sub>τ1</sub>/τ²<sub>2</sub>)+(σ²<sub>τ2</sub>×τ²<sub>1</sub>/τ²<sub>2</sub>)]<sup>1/2</sup>, where R=τ<sub>1</sub>/τ₂ and where values of σ are the respective s.d.

Table 3 The effect of amphotericin B on the muscle and liver plasma per membrane ATPase of normal and dystrophic chicks (µmol Pi per mg protein per 20 min)

	C	Control membrane		Am	photericin B treat	ed
Condition	$Na^+-K^+-Mg^{2+}$	+Ouabain	$+Ca^{2+}$	$Na^+-K^+-Mg^{2+}$	+Ouabain	+ Ca <sup>2+</sup>
Tissue Muscle					•	
Normal	$1.86 \pm 0.01$	$1.83 \pm 0.01$	$2.43 \pm 0.04$	$2.07 \pm 0.04$	$2.26 \pm 0.02$	2.40 : 0.02
Dystrophic	$1.21 \pm 0.05$ P < 0.01	$0.97 \pm 0.01$ P < 0.01	$1.34\pm0.01$ $P < 0.01$	$1.21 \pm 0.01$ $P < 0.01$	$1.23 \pm 0.03$ $P < 0.01$	$1.52 \pm 0.02$ P < 0.01
Liver						( 0 0 10
Normal	$10.7 \pm 0.19$	$8.9 \pm 0.16$	$9.1 \pm 0.20$	$10.1 \pm 0.19$	$6.4 \pm 0.08$	6.2 : 0.12
Dystrophic	$6.4 \pm 0.23$ P < 0.01	$6.7 \pm 0.07$ P < 0.01	$6.6 \pm 0.55$ P < 0.01	$6.4 \pm 0.10$ P < 0.01	$7.3 \pm 0.16$ P < 0.01	6.2 - 0.16 NS

Plasma membrane of muscle and liver and their treatment with amphotericin B, were as described in Table 2. ATPase activity was assayed by incubating 100 µg protein per assay tube for 20 min at 37° C in 40 mM imidazole, 10 mM Tris-HCl, 2 mM Mg (pH 7.4) in the presence of 100 mM NaCl and 10 mM KCl to which either 0.1 mM ouabain or 0.2 mM calcium were added. The ATP concentration was 1 mM. Inorganic phosphate was determined colorimetrically using the ammonium molybdate reaction<sup>20</sup>. The results represent the mean ± s.d. of three determinations. Statistical significance of the differences between means was determined using Student's t test.

The Na+-K+-Mg<sup>2+</sup>-ATPase activity of the dystrophic chick muscle sarcolemma was reduced relative to control and was less stimulated by calcium, as previously observed<sup>5</sup>. Of particular interest were the substantial differences observed in the ATPase activity of the liver plasma membranes. As in muscle, the basal level was lower in dystrophic chicks. Ouabain moderately inhibited the ATPase activity of the normal membrane and slightly enhanced that of the dystrophic one. This difference became more prominent after amphotericin B treatment, when ouabain stimulation was clearly observed in the dystrophic membrane. ATPase stimulation by ouabain has also been observed in erythrocyte membrane from DMD patients<sup>7</sup>. Calcium (10<sup>-4</sup> M) had a similar effect to that of ouabain.

Although it is difficult at present to fit the various differences between the membrane of normal and diseased chicks into a single comprehensive molecular model, the findings support the hypothesis of a generalised membrane defect as an underlying genetic disorder in muscular dystrophy, making it a possible eukaryote membrane mutant. The effects of amphotericin B and digitonin are consistent with the possibility that the basis of this defect is a disorder in lipid metabolism and/or lipid assembly into the plasma membrane.

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### Voltage clamp studies of glutamate synapse

THERE is considerable evidence that L-glutamate is the excitatory transmitter both at the insect and crustacean neuromuscular junction<sup>1,2</sup>, and also in the vertebrate central nervous system<sup>3</sup>. Here we report the use of the voltage clamp technique to compare the reversal potentials of the conductance increase produced by the excitatory transmitter and the application of L-glutamate at the insect neuromuscular junction. We also investigated the ionic basis of the L-glutamate conductance increase using voltage clamping. These experiments could be carried out accurately because changes in resistance of the electrically excitable membrane caused by changes in membrane potential or by different ionic media do not affect the amplitude of the excitatory junctional currents or glutamate currents.

We studied the extensor tibiae muscle of the metathoracic leg of the locust Schistocerca gregaria. The muscle fibres were clamped using a two microelectrode method, with a potential recording electrode connected to a current passing electrode through a high input impedance amplifier and a feedback amplifier. The smallest muscle fibres which have a length of 1.5 mm and a diameter of 100 µm could be clamped with only 2% error using this technique.

In normal saline (NaCl 170.0, KCl 10.0, CaCl<sub>2</sub> 2.0, HEPES buffer 10.0 mM 1<sup>-1</sup>; pH 6.8), resting potentials of 55-60 mV were recorded. The neurally-evoked responses were reduced in amplitude by the addition of 40 mM Mg to the saline. Stimulation of the excitatory axon to the muscle with a suction electrode produced a transient depolarisation in the unclamped fibre, the excitatory junctional potential (e.j.p.). In the clamped fibre, nerve stimulation produced an inward current, the excitatory junctional current (e.j.c.). An e.j.p. and e.j.c. are shown in Fig. 1. The e.j.c.s had a maximum amplitude of  $1 \times 10^{-6}$  A and an average rise time of 3.9 ms, a much shorter time course than the e.j.p. L-glutamate was applied to the muscle iontophoretically, using high resistance electrodes which obviated the use of braking currents. Iontophoresis of L-glutamate on to the junctional region of the muscle produced a transient inward current of maximum amplitude  $1\times10^{-7}$  A. The rise time of the glutamate currents varied between 20-100 ms, depending on the distance of the iontophoretic electrode from the junction.

Reversal potentials were determined by clamping the e.j.c. and glutamate current at different membrane potentials. The currents were reduced in amplitude as the membrane was depolarised. Actual reversal of the sign of the currents was difficult to achieve because of muscle contraction. Consequently, most reversal potentials were obtained by extrapolation. A linear relationship was obtained for the e.p.c.s and the glutamate currents between 0 mV and 60 mV. The reversal potential of the e.j.c. was  $+3.4 \pm 5.2$  mV (mean  $\pm$  s.d., eight experiments in eight muscles), and the reversal potential of the glutamate current was  $+2.7 \pm 2.4$  mV (mean  $\pm$ s.d., eight experiments in eight muscles). Sometimes a slow outward current was observed following the inward glutamate current when the membrane was depolarised. This is probably due to stimulation of extrajunctional glutamate receptors causing an increase in permeability to Cl (ref. 4).

Takeuchi and Onodera<sup>5</sup> have recently obtained a close agreement between the reversal potentials of the e.p.c. and the glutamate current at the crustacean neuromuscular junction. In insects, reversal values of between -10 mV and -25 mV have been obtained for the glutamate potential and the miniature excitatory postsynaptic potentials at the locust neuromuscular junction<sup>8</sup>. Moreover, reversal values of +6 mV for the excitatory junctional potential and +7 mV for the glutamate potential have also been obtained by reversal of sign at the locust neuromuscular junction (S. G. Cull-Candy and P.N.R.U., unpublished).

Perfusion with Na-free (choline Cl substituted) saline caused a 90-95% reduction in the amplitude of the junctional glutamate current. The reversal potential in the Na-free saline varied between -10 and -15 mV. Changes in the K concentration of the saline between 0 mM and 20 mM did not alter the reversal potential of the glutamate current. A reduction in the external K from 10 to 0 mM doubled the amplitude of the current, however, the opposite of that expected if K contributed to the current. The reversal potential of the junctional current was also unchanged in Cl free saline. Increasing Ca from 2 to 50 mM in normal saline reduced the amplitude of the glutamate current by about 90%, but the small glutamate current which can be recorded in Na-free saline increased in amplitude by up to six times when Ca was increased from 2 to 50 mM.

As the reversal potentials for the e.p.c. and the glutamate current were close to zero, it seemed likely that they were the resultants of a simultaneously occurring inward Na and outward K or Cl currents, as the equilibrium potentials for Na,

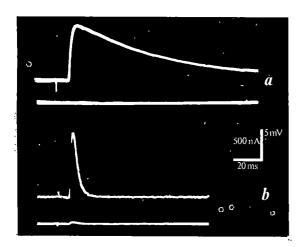


Fig. 1 a, e.j.p. Recorded intracellularly from an extensor tibiae muscle fibre of the locust. b, Upper record, (e.j.c.) recorded from the same muscle fibre with the membrane potential held at the resting level using voltage clamping; lower record, clamped membrane potential. Only a small deviation of 0.1-0.2 mV is seen after nerve stimulation.

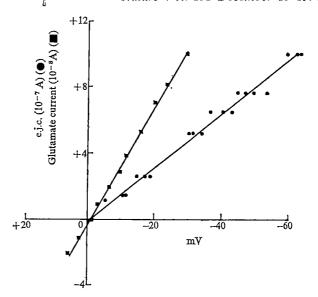


Fig. 2 Relationship between the membrane potential and the amplitude of the e.j.c. (●) and glutamate current (■).

K and Cl in locust muscle have been theoretically calculated as +43 mV, -61 and -50 mV, respectively. The e.p.c.s and acetylcholine currents at the vertebrate neuromuscular junction have reversal potentials between -10 mV and -20 mV, and are generated by simultaneous inward Na currents and outward K currents.

The results of our study, however, suggest that the glutamate current consists only of an inward current carried by Na, with Ca perhaps making a small contribution. K and Cl do not seem to carry any outward current, as alteration of external K and Cl does not change the reversal potential. The absence of any outward current is difficult to explain if the theoretically calculated  $E_{\rm Na}$  for locust muscle is correct. Perhaps the intracellular or extracellular concentrations of Na at the synaptic region are different from the non-synaptic regions, so that  $E_{\rm Na}$  at the synaptic region is close to 0 mV. It is also surprising that a large change in the concentration of external Na only causes a small shift in the reversal potential. This may be due to Ca, which has a very positive equilibrium potential, carrying a greater proportion of the current as external Na is lowered.

Another explanation of the results is that both Na and K do participate in the glutamate current, but the sodium and potassium conductance ratio,  $\Delta g_{\rm Na}/\Delta g_{\rm K}$  varies as the ionic media is altered so that the reversal potential remains at or near zero. Thus as external Na is reduced,  $\Delta g_{\rm Na}/\Delta g_{\rm K}$  and  $\Delta g_{\rm Ka}$  increases, while as external K is reduced,  $\Delta g_{\rm Na}/\Delta g_{\rm K}$  decreases. At the vertebrate muscle end-'plate,  $\Delta g_{\rm Na}/\Delta g_{\rm K}$  stayed constant with most changes in the ionic media, but did decrease markedly when the external K was increased above 10 mM (ref. 9).

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# Synapse formation between clonal muscle

cells and rat spinal cord explants

Although functional neuromuscular junctions have been reported between cultured muscle and spinal cord explants<sup>1-8</sup> or dissociated spinal cord cells<sup>4</sup>, further studies have been hampered because primary cultures represent a mixture of cell types of unknown function. Harris *et al.*<sup>5</sup> tackled this problem by coculturing a neuronal cell line (C1300) from a mouse sympathetic ganglion cell<sup>8,7</sup> with a rat skeletal muscle cell line (L6)<sup>8</sup>. They found regions of high acetylcholine (ACh) sensitivity at points of contact between nerve and muscle, indicating interaction between nerve and muscle<sup>5,9</sup>. So far no functional synapses have been observed in this system. We now report that the L6 muscle cell line can form functional synapses with a competent nerve.

The L6 cell line culture and intracellular recording techniques for monitoring the membrane potential were as reported previously<sup>5,9,10</sup>. Electrophysiology was done at room temperature (20°–24° C) in the following saline (NaCl, 142.6 mM; KCl, 5.6 mM; CaCl<sub>2</sub>, 10 mM; HEPES (N-2 hydroxyethylpiperazine-N'-2-ethansulphonic acid)-NaOH, 5 mM, pH 7.4). In some experiments electrode noise was minimised to 100  $\mu$ V peak-to-peak by using a rather low resistance pipette (50 M $\Omega$ ) and a low pass filter (time constant 0.5 or 1 ms).



Fig. 1 A microscope picture showing a few nerves making contact with an L6 myotube. The bar at the right corresponds to  $50~\mu m$ .

Spinal cords were dissected from 10–20-d-old rat foetuses. Connective tissue was removed and the cord was cut into pieces, about  $0.1 \text{ mm}^3$ , with a pair of needles. Several pieces were placed on a monolayer of myotubes with 0.1–0.5 ml of culture medium and incubated for 1–3 h to allow the explant to stick to the underlying cells. Then modified Eagle's medium<sup>11</sup> supplemented with 10% foetal calf serum was added. During the next few days processes grew out of the explant and several rapidly dividing cells began to be seen, probably fibroblasts and myoblasts. To reduce their proliferation d-arabinofuranosylcytosine  $(5\times10^{-5} \text{ M})$  was added after 3–5 d (ref. 4). After 5–7 d the cultures were returned to the original medium.

At various stages of coincubation the myotubes were penetrated with microelectrodes to record miniature end-plate potentials (m.e.p.p.s.). Figure 1 shows a typical nerve muscle pair in which the muscle is contacted by a few nerves. The m.e.p.p.s. were first observed after 7 d, usually a few per minute. After 2 weeks many myotubes produced m.e.p.p.s. 6-60 per min (Fig. 2 shows sample traces).

The cholinergic nature of these m.e.p.p.s. was demonstrated by applying d-tubocurarine  $(0.3-10\times10^{-7} \text{ M})$ . The half blocking dose was about  $4\times10^{-7} \text{ M}$ ; larger than that found for rat neuromuscular junction  $(3\times10^{-8} \text{ M})$  but similar to that found

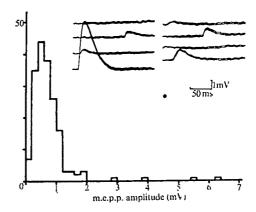


Fig. 2 Amplitude histogram of m.e.p.p.s. Sample recordings of m.e.p.p.s.

for denervated rat muscle<sup>12</sup> and for the potential changes evoked by iontophoretic application of ACh in the L6 cell line<sup>13</sup>.  $\alpha$ -Neurotoxin isolated from an Indian cobra, *Naja naja*, also blocked m.e.p.p.s. at a concentration of  $4 \times 10^{-7}$  g ml<sup>-1</sup>; this is similar to the concentration required to block the response of L6 myotubes to iontophoretically applied ACh<sup>14</sup>.

To determine whether these synapses contain functional acetylcholinesterase the effect of an acetylcholinesterase inhibitor was examined. Edrophonium chloride (Tensilon, Hoffman La Roche<sup>15,16</sup>) at 3.8 to 100 µM did not increase the amplitude, the time-to-peak or the half-decline time of m.e.p.p.s. recorded in cultures after 15–31 d of coincubation. This result agrees with observations on similar systems. Koenig<sup>17</sup> has reported histochemical evidence, however, for the presence of acetylcholinesterase in this system.

The m.e.p.p. amplitude and time course varied over a large range. For example, in the fibre shown in Fig. 2, the amplitudes varied from 0.2 mV to 6.3 mV, the time-to-peak from 6 to 26 ms and the half-decline time from 8.5 to 31 ms. The amplitude histogram which was obtained in the presence of tetrodotoxin  $(6 \times 10^{-6} \text{M})$  to block end-plate potentials (e.p.p.s.), is skewed (in Fig. 2 the coefficient of skewness is 4.6). A similar skewed distribution was found in neuromuscular junctions formed in vitro<sup>4</sup> and regenerating neuromuscular junctions<sup>18,19</sup>.

The mean m.e.p.p. amplitude was also variable from cell to cell. There was a roughly linear correlation between the steady input resistance of the L6 myotube and the mean amplitude of the m.e.p.p.s. recorded in that myotube (in

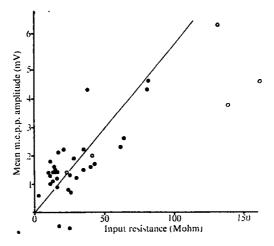


Fig. 3 Correlation between the steady state input resistance and the mean m.e.p.p. amplitude. The m.e.p.p. amplitude is corrected for the resting potential by multiplying by a factor 64/(V-5) (where V is the absolute value of the resting potential in mV). The standard resting potential is taken as -69 mV and the equilibrium potential for the m.e.p.p. was assumed to be -5 mV (ref. 13). Open circles were obtained in preparations treated with La (about 0.1 mM). The straight line is drawn by eye.

Fig. 3 the correlation coefficient is 0.83). The slope of the regression line for L6 myotubes is 0.05 mV for 1 M $\Omega$  of input resistance (Fig. 3). This is 3.7% of that found for the frog (1.33 mV per  $M\Omega$ )<sup>15</sup>. The maximum current during the m.e.p.p.s could be estimated from the maximum rate of rise of the m.e.p.p.s. and the input capacitance of the postsynaptic cells ignoring the current flowing through the resistive component 20. The maximum rate of rise of the average sized m.e.p.p. in the cell shown in Fig. 2 was 0.19 V s<sup>-1</sup> while the input capacitance obtained by analysing the time course of the membrane potential after applying a small amount of hyperpolarising current, was 1.6 nF. The maximum current was then  $0.3 \times 10^{-9}$  A. Assuming an equilibrium potential for the m.e.p.p.s. of -5 mV, the conductance change during average sized m.e.p.p. was calculated to be  $7.6 \times 10^{-9}$  mho (3.5 to  $10.0 \times 10^{-9}$  mho in six cells). This is 5% of the conductance increase measured in frog neuromuscular junction (1.5×10<sup>-7</sup> mho)<sup>21</sup>. This small conductance increase during m.e.p.p.s. could be explained, for example, by a small amount of ACh in one packet or by a low ACh receptor density in the subsynaptic membrane. Patrick et al.14 found 100 receptors per µm2 in non-innervated L6 myotubes, 0.3 to 1.4% of that found at adult end-plates in mammalian striated muscle22-24.

When a nerve bundle in contact with a myotube was stimulated with a suction electrode (tip diameter about 5  $\mu$ m, filled with saline), an e.p.p. was evoked (Fig. 4a). The nerve was stimulated about 50  $\mu$ m from the nerve-muscle contact. There was a relatively long latency (7 ms) between the nerve stimulation and the onset of the e.p.p. Although the e.p.p.s. sometimes failed (seven failures out of 22 consecutive stimulations), no discrete steps of e.p.p. amplitude were seen.

Addition of KCl (20 mM final concentration) did not change the m.e.p.p. frequency although it depolarised the muscle (Fig. 4b). This was the case even in nerve muscle pairs where e.p.p.s. could be evoked by nerve stimulation.

High tonicity causes a large increase in the m.e.p.p. frequency at adult synapses<sup>25</sup> but the addition of sucrose (400 mM) did not increase the m.e.p.p., frequency in this system. A similar

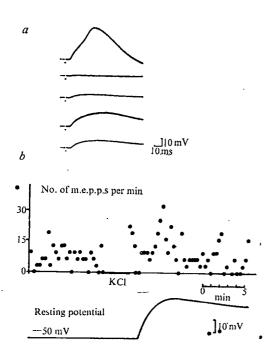


Fig. 4 a, e.p.p.s evoked by stimulating the nerve bundle. Stimulus intensity was kept constant. The top record has a small regenerative potential superimposed on the e.p.p. b, The effect of KCl on m.e.p.p. frequency. A small amount of KCl (1 M) was added to the bath during the time indicated by a bar to a final concentration of 20 mM. The lower trace is the membrane potential of the muscle cell showing the time course of depolarisation.

insensitivity to high KCl and high tonicity was found in newly formed Xenopus neuromuscular junctions in vitro<sup>3</sup>. In several cases lanthanum (about 0.1 mM) increased the m.e.p.p. frequency for a few minutes and then the m.e.p.p.s. disappeared. In most cases, however, lanthanum ions suppressed the m.e.p.p.s without any apparent stimulation. In adult frog neuromuscular junctions lanthanum ions increase the m.e.p.p. frequency for hours before the m.e.p.p.s. finally disappear<sup>28</sup>.

The electrophysiological properties of the synapses formed between the L6 cell line and spinal cord explants are similar to those reported in chick primary cultures<sup>1, 2, 4</sup>.

Thus a clonal muscle cell line adapted to continuous cell culture can make a synapse with a suitable nerve. Previous failures to detect functional synapses between L6 myotubes and the neuroblastoma cell line must have been due to the incompetence of the nerve. The availability of many independently derived nerve, glia and muscle cell lines<sup>27,28</sup> will make possible an investigation of the requirements of synapse formation.

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# Rapid reversal of internal Na<sup>+</sup> and K<sup>+</sup> contents of synaptosomes by ouabain

THERE is evidence that a number of drugs which have profound effects on the central nervous system (CNS) may do so by altering membrane bound (Na++K+)-dependent ATPase and/or Na+ pump activities. For example, ouabain which is an inhibitor of neuronal (Na++K+) dependent ATPase¹ and Na+ pump activity², can impair short term memory in small doses³ and produce convulsions when larger doses are administered intraventricularly⁴.

It is generally considered that the synaptic region of the neurone is especially sensitive to the actions of drugs. In the case of drugs which modify  $(Na^++K^+)$ -dependent ATPase activity, it is interesting that this enzyme is found in abundance in synaptosomal preparations. Moreover, synaptic terminals in the CNS, which are generally 1 µm or less in diameter, would have very small internal volumes so that alterations in Na+ pump activity might be expected to cause relatively rapid changes in the concentrations of Na+ and K+ inside the terminals. In contrast, the squid giant axon, with its much larger internal volume, and therefore larger reservoir of K+, seems to be fairly resistant to Na+ pump inhibition insofar as it maintains its resting potential even after the Na+ pump has been inhibited by ouabain or metabolic inhibitors for over an hour<sup>2,7</sup>.

We have found that ouabain reversed the normal  $Na^+$  and  $K^+$  contents of synaptosomes within 1 min of exposure to the drug, indicating that presynaptic terminals might be particularly sensitive to drugs which influence  $Na^+$  pump activity.

Synaptosomes were prepared from the brains of 2 male Wistar rats (200–250 g) and isolated on sucrose density gradients as described previously<sup>8</sup>, before being finally resuspended with 0.5 ml of ice-cold 0.32 M sucrose. The synaptosomes were then incubated in media containing NaCl, KCl, CaCl<sub>2</sub>, MgCl<sub>2</sub> and Tris-HCl and the effects of 1 mM ouabain on the Na<sup>+</sup> and K<sup>+</sup> contents determined as described in Fig. 1.

Figure 1a shows that 1 mM ouabain caused a significant reduction in synaptosomal  $K^+$  content (paired t test) at 30 s with a maximal effect by 1 min. Synaptosomes gained Na<sup>+</sup> during incubation (Fig. 1b) but ouabain increased further the Na<sup>+</sup> content after 1 min (paired t test) with a maximal effect achieved after 3 min. At time zero, the  $K^+/Na^+$  ratio was 1.51 for the control synaptosomes (Fig. 1c) which compares favourably with the value of 1.78 found previously for synaptosomes immediately after isolation on a discontinuous sucrose density gradient<sup>12</sup>. During incubation for 1 min, this ratio decreased somewhat to 0.93 for the controls but ouabain completely reversed the  $K^+/Na^+$  ratio to 0.39 by 1 min.

These results clearly indicate that ouabain can very quickly reverse the Na<sup>+</sup> and K<sup>+</sup> contents of synaptosomes. If, as much evidence suggests, synaptosomes are functionally intact presynaptic nerve terminals<sup>13-15</sup>, and if one can extrapolate from these *in vitro* experiments to the *in vivo* situation, it would seem that alterations of synaptic Na<sup>+</sup> pump activity might very quickly change the internal Na<sup>+</sup> and K<sup>+</sup> contents of synaptic terminals, possibly because of the very small internal volumes of the terminals. This rapid change in Na<sup>+</sup> and K<sup>+</sup> content could in turn affect the resting membrane potential<sup>7</sup>, the generation of action potentials<sup>7</sup>, the release and reuptake of neurotransmitters<sup>6,16</sup> and even protein synthesis<sup>17,18</sup> in the presynaptic terminal. Thus, one might expect synaptic transmission to be particularly susceptible to alterations in neuronal Na<sup>+</sup> pump activity.

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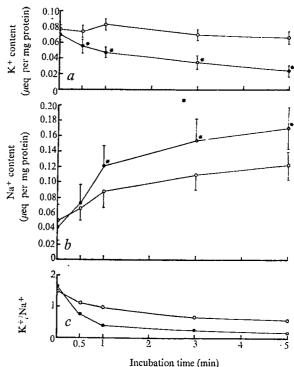


Fig. 1 Time course for the effect of ouabain on the Na<sup>+</sup> and K<sup>+</sup> contents of synaptosomes. Incubation medium (2 ml) containing 120 mM NaCl, 3 mM KCl, 3 mM MgCl<sub>2</sub>, 2 mM CaCl<sub>2</sub>, 20 mM Tris-HCl(pH 7.4) and sometimes 1 mM outbain were prewarmed to 37° C under air in a 10 ml conical flask in a shaking incubator. To avoid any effects of ouabain on glucose uptake which might indirectly influence synaptosomal Na<sup>+</sup> pump activity, glucose was omitted from the incubation medium. At zero time, 0.10 ml of synaptosomal suspension was added and the incubation continued; the reaction was stopped by removing and filtering 0.5 ml of medium on a Millipore filter (0.45  $\mu$ m pore size and 2.5 cm diameter) and washing with 10 ml, ice-cold medium containing 260 mM sucrose, 3 mM MgCl<sub>2</sub>, 2 mM CaCl<sub>2</sub> and 20 mM Tris-HCl (pH 7.4). This washing procedure, which took approximately 20 s, did not seem to rupture the synaptosomes. A 1.5 cm diameter disk containing the synaptosomes was cut out from the filter and Na<sup>+</sup> and K<sup>+</sup> were eluted overnight with 0.6 ml of 15 med l<sup>-1</sup> lithium diluent solution (Instrumentation Laboratory, Inc., lithium nitrate standard). This hypotonic treatment releases the Na<sup>+</sup> and K<sup>+</sup> contained in osmotically sensitive compartments of the synaptosomes. The Na<sup>+</sup> and K<sup>+</sup> contents of the diluent were determined with an IL model 143 flame photometer and the values corrected for the Na+ and K+ eluted from the filter itself by subtracting the amount of Na+ and K+ released into diluent from filters which had been treated as above except that no synaptosomes were present in the incubation medium. Na<sup>+</sup> contamination due to trapping of incubation medium in the extracellular space of the synaptosomes on the filters was determined in the following manner. Synaptosomes were incubated as before but 0.01 ml of 35S-sulphate (1 mCi ml<sup>-1</sup>) as an extracellular marker<sup>8</sup> was added immediately before filtering and washing. The radioactivity in the filter was determined by scintillation counting and compared with the radioactivity-present originally in 0.01 ml of the <sup>35</sup>S-sulphate solution to give the percentage contamination of the filter and synaptosomes by incubation medium. This estimate was performed for various concentrations of synaptosomal protein including a blank which contained no synaptosomes. The latter value was subtracted from the values obtained with synaptosomes present to give the amount of contamination in the extracellular space and these values were used to calculate the percentage contamination by incubation medium. The extrasynaptosomal Na<sup>+</sup> contamination from the incubation medium was calculated and subtracted from the total Na<sup>+</sup> content of the synaptosomes on the filter to give the actual Na<sup>+</sup> content of the synaptosomes. Contamination by extracellular  $K^+$  was not corrected because of the small amounts of  $K^+$  present in the original incubation media. The final ion K<sup>+</sup> present in the original incubation media. The final for contents were expressed as µeq ion per mg synaptosomal protein. Protein was determined by the Hartree<sup>10</sup> modification of the method of Lowry et al.<sup>11</sup>, <sup>35</sup>S-sulphate was supplied by New England Nuclear Corp., Ltd., Montreal and ouabain by the Sigma Chemical Co., St Louis. a, K<sup>+</sup> content; b, Na<sup>+</sup> content; c, ratio of K<sup>+</sup>: Na<sup>+</sup>; O, control; •, 1 mM ouabain. Each point represents the mean ±s.e. of 12 determinations (six experiments).

\*Ouabain treatment is significantly different from the control Ouabain treatment is significantly different from the control (P < 0.05, paired r test).

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### Coordinate and differential in vitro syntheses of two RNA polymerase subunits

THE B subunit of Escherichia coli RNA polymerase—one of three subunit types  $(\alpha, \beta, \beta')$  in the enzyme core structure—is specified by a genetic locus, rif (ref. 1). Specialised transducing phages which carry the rif region have been isolated<sup>2,3</sup>. If DNA is extracted from these phages, it can be used to direct the synthesis of the B subunit in vitro in a coupled transcription/ translation system<sup>4,5</sup>. It has been suggested that other RNA polymerase structural genes may also be in this region of the chromosome<sup>6</sup>. Recent genetic evidence shows that the  $\beta$  and  $\beta'$ subunits are produced from a single transcriptional unit in vivo7. DNA extracted from three independently isolated rif transducing phages however, failed to direct the synthesis of any detectable \( \beta' \) molecules in vitro in the coupled systems previously described<sup>4.5</sup> (Fig. 1a). Here I describe a coupled system which synthesises  $\beta$  and  $\beta'$  in vitro with equal efficiency from rif transducing phage DNA.

The new method involves the use of modifications in the preparation of bacterial extracts (S30s) similar to those developed by Wilcox et al. who used them to maximise the synthesis of arabinose isomerase in vitro using λh<sub>80</sub>dara template DNA<sup>8</sup>. The data presented in Fig. 1 provide strong evidence that both  $\beta$  and  $\beta'$  subunits can be synthesised in the modified system, using  $\phi 80d_7 rif^7$  DNA as a template. Two newly-synthesised proteins migrate upon electrophoresis in sodium dodecylsulphate polyacrylamide gels to positions coincident with those of marker bands for  $\beta$  and  $\beta'$ . These proteins are specifically precipitated by serum prepared against highly purified E. coli RNA polymerase (Fig. 1). Thus these proteins are antigenically related to purified RNA polymerase, and have apparently identical molecular weights to those of the  $\beta$  and  $\beta'$  subunits.

If the proteins made in vitro are indeed RNA polymerase subunits, then DNA-containing mutations which alter polymerase structural gene expression in vivo should also affect the synthesis of the proteins in vitro. We have previously described a class of mutations (rif° mutations) which are candidates for  $\beta$  structural gene mutations. The rif° mutations

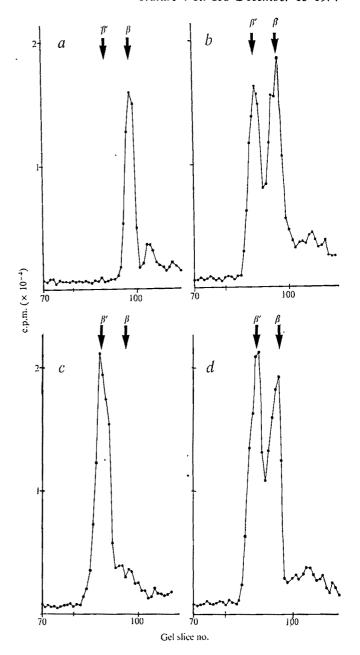


Fig. 1 Cell free protein synthesising systems containing type 1 or type 2 S30s were run as previously described with the addition of 15 mg ml<sup>-1</sup> of polyethylene glycol and 0.2  $\mu$ mol disphosphopyridine nucleotide. 0.1 ml systems contained 0.3 mCi of <sup>3</sup>H-L-leucine, specific activity 60 Ci mmol<sup>-1</sup>, 10  $\mu$ g of template DNA, and either 2 nmol E. coli transfer RNA from a non-suppressing (su<sup>-</sup>) strain or 2 nmol of tRNA enriched for the suppressor SuIII tRNA<sup>10</sup>. After incubation, the systems were diluted to 0.4 ml with buffer incubation, the systems were diluted to 0.4 ml with buffer having the same salt composition as the cell free system<sup>4</sup>, mixed thoroughly, and centrifuged at 4° C at 40,000g for 15 min. The supernatant was decanted into a tube containing 20  $\mu$ I of antiserum prepared against highly purified RNA polymerase. This serum is highly specific for the participapolymerase. This serum is nightly specific for the participation of RNA polymerase and its subunits (S.A., unpublished). After 12 h at 4° C the precipitate was collected by centrifugation at 40,000g for 30 min. The pellet was resuspended in 50  $\mu$ l of sample buffer<sup>4</sup>, and was run on sodium dodecylsulphate polyacrylamide gels as previously described<sup>4</sup>, except that 2 mm thick slab gels<sup>14</sup> were substituted for the tubular that 2 mm thick stab gels" were substituted for the fibrilar ones. The gels were stained, sliced, and counted for radio-activity as previously described. The arrows show the position of the stained marker bands for β and β'. a, Type 1 S30, Φ80d<sub>7</sub>rif' DNA; b, type 2 S30, Φ80d<sub>7</sub>rif' DNA; c, type 2 S30, Φ80d<sub>7</sub>rif'-B115 DNA; d, type 2 S30 plus suIII enriched tRNA, Φ80d<sub>7</sub>rif'-B115 DNA.

Table 1 The synthesis of arabinose isomerase and  $\beta$  galactosidase in vitro using type 1 and type 2 S30 cell extracts

	Arabinose acti		β-galactosidase* activity		
DNA template λh <sub>80</sub> dara λh <sub>80</sub> d lac	Type 1 S30 < 0.04 < 0.04	Type 2 S30 3.2 < 0.04	Type 1 S30 <0.01 12		

Type 1 S30 cell extracts were prepared as described<sup>11</sup> by lysis in an Aminec French Pressure Cell at 5,000 pounds per square inch pressure. Cell extracts prepared by alumina grinding, were also used and had identical properties to type 1 S30s. Type 2 S30s were prepared as described but with French Cell pressures further reduced, so that 30% of the bacteria remain unbroken (600–1,000 pounds per square inch). Both types of S30 were active for protein synthesis as shown by their ability to promote  $\beta$ -galactosidase synthesis from  $\lambda h_{80}d$  lac template DNA (Table 1). The protein synthesis mixtures<sup>8</sup> were incubated for 80 min at 35.5° C with µg ml<sup>-1</sup> of template DNA. Arabinose isomerase activity was dependent on *de novo* synthesis of the regulatory product directed by the ara C gene<sup>8</sup>. The C gene product can also be made using type 1 S30's (ref. 13).

\*Arabinose isomerase assays<sup>8</sup> and β-galactosidase assays<sup>9</sup> are expressed as μmol of substrate converted h<sup>-1</sup> ml<sup>-1</sup> of *in vitro* system; 1 ml assay mixtures contained 0.1 ml of the in vitro system.

eliminate the expression of the rifampicin sensitivity phenotype, which is known to be associated with the β structural gene<sup>1</sup>. I have transfered a rifo mutation of the chain terminating class,  $rif^{\circ}$ -B115  $_{amber}$ , from the host chromosome to the  $\phi 80d_{7}rif^{\circ}$ transducing phage by recombination (Austin, in preparation). DNA extracted from the recombinant phage, φ80d<sub>2</sub>rif°-B112, produces no  $\beta$  peak *in vitro* but still directs  $\beta'$  synthesis (Fig. 1c). If purified E. coli transfer RNA, enriched for mutant suIII tRNA is added to the system<sup>10</sup> the synthesis of the β protein is restored (Fig. 1d). Thus rif o-B115 is a suppressible nonsense mutation which eliminates the expression of the β gene. As it has no apparent effect on  $\beta'$ , a gene distal to the  $\beta$  gene in the same operon7, rif°-B115 is probably a β structural gene mutation.

The difference in results obtained with the in vitro system by changing the type of \$30 used is notable. The use of the modified (Type 2) S30 is, within the limits of detection of my assays, an absolute requirement for the synthesis of  $\beta'$  from φ80d<sub>7</sub>rif<sup>\*</sup> DNA, and of arabinose isomerase from φ80dara DNA (Fig. 1, Table 1). The original, however, (Type 1), S30s (ref. 11) suffice for the synthesis of  $\beta$  subunit from  $\phi 80d_2 rsf^{\tau}$ (Fig. 1). A second product of the ara operon, ribulokinase, can also be made efficiently using type 1 S30s (ref. 12). Thus the in vitro system can show a striking discrimination between two products which at least in vivo, are the products of a single messenger RNA. Both the rif and ara operon show this effect. This suggests that the effect may have some general relevance to the production of proteins from polycistronic messengers. I am now investigating whether the discrimination occurs at the level of transcription or translation. The present system should provide an assay for attempts to purify a discriminating agent from S30 extracts.

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### Alteration of melanocytes by DNA in White Plymouth Rock chickens

SINCE 1944 when it was first demonstrated that the pneumococcal transforming principle was DNA1, several investigators have reported evidence suggesting genetic alteration of warmblooded animal cells by DNA-mediated transformations<sup>2-7</sup>. Attempts to transform the plumage coloration characters in White Plymouth Rock embryos have been unsuccessful<sup>8</sup>. The genetically lethal melanocytes in the White Plymouth Rock chicken do not appear abundantly or thrive for long in the feather germ. They degenerate before they can deposit a significant number of melanin granules in the feather-forming cells. Using nucleoprotein from the Harco chicken, a standard cross between the Barred Plymouth Rock hen and the Rhode Island Red rooster, I have extended the life span of the melanocytes in White Plymouth Rock embryos.

Nucleoprotein was extracted from about 1 g (wet weight) of feather anlage, from 10-d-old Harco chicken embryos. The tissue was suspended in a solution of 0.25 M sucrose (pH 7.4) and 1 mM EDTA at a 1:9 (w/v) ratio of tissue to sucrose. The mixture was homogenised in a tissue grinder with an inhibitor of DNase (NaF, 0.044 M). Nucleoprotein was extracted with 2 M sodium chloride as described by Zamenhof10, except that the mixture was left at 4° C for only 2 h to avoid damage to DNA. The nucleoprotein was suspended in 4 ml of normal saline and divided into two parts: one part was left untreated and to the second part, crystalline pancreatic DNase (0.1 mg ml<sup>-2</sup>) and MgCl.6H<sub>2</sub>O (5 mM) were added. The preparations were incubated at 25° C for 1.5 h and then 0.04 ml was injected into small extra-embryonic veins of 5-d-old



Fig. 1 Light micrograph of melanin granules in the feather anlage from the head of a 14-d-old White Plymouth Rock embryo, which was injected with DNA on the day 5 of incubation  $(\times 100)$ .

White Plymouth Rock embryos. By this time the melanoblasts have migrated from the neural crest into the skin<sup>11</sup>.

Twelve of the nineteen embryos injected with nucleoprotein survived beyond day 14 of incubation. Starting from this time and continuing until birth, nine of these embryos were examined at 2-d-intervals. The host embryo's melanocytes would have degenerated by this time if left undisturbed. The remaining three embryos were allowed to hatch. Patches of pigmented feathers were found on five of the embryos and on the juvenile down of one newborn chick (Fig. 1). Their coats were otherwise white.

The active component in the transforming material seems to be DNA because no DNase-treated preparation induced pigmented feathers. Thus treatment of White Plymouth Rock embryos with DNA carrying a marker for full-lifed melanocytes resulted in the development of pigmented feathers in six out of twelve cases.

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### In vitro maturation of precursors of 5S ribosomal RNA from Bacillus subtilis

THE ribosomal RNA (rRNA) molecules both of prokaryotes and of eukaryotes are not the immediate products of DNA transcription. Rather, precursors of the mature RNA species undergo a series of nucleotide modifications and/or cleavage events 1,2. Only limited information is available concerning the mechanics of and rationale for post-transcriptional tailoring of RNA because of the large sizes of the precursor molecules and consequent difficulty in evaluating their nucleotide sequences.

By virtue of its small size (about 120 nucleotides), 5S rRNA is convenient for study but its maturation is not always as complex as that affecting the high molecular weight rRNA molecules (16S and 23S rRNA, in prokaryotes). In Escherichia coli, for example, the precursor of 5S rRNA (p5) is converted to the mature molecule (m5) by an exonuclease<sup>3,4</sup> rather than a specific endonuclease, which probably is responsible for maturation of 16S and 23S rRNA. In Bacillus subtilis, however, metabolism of 5S rRNA apparently mimics that of the larger rRNA molecules. In the absence of protein synthesis (presence of chloramphenicol) B. subtilis accumulates two precursors of 5S rRNA, designated p5<sub>A</sub> (180 nucleotides) and p5<sub>B</sub> (150 nucleotides). Both are converted independently to m5 rRNA, which in this organism is composed of 118 nucleotides. We here report that maturation of p5A and p5B is effected in vitro by a partially purified, specific cleavage enzyme, which we denote as RNase M5.

Incubation of purified 32P-labelled p5A and p5B with partially purified RNase M5 from B. subtilis yields several products. Figure 1 displays polyacrylamide gel electrophoretograms of the

purified substrates (Fig. 1a and b) and the products of the cleavage reaction (Fig. 1c and d). The  $p5_A$  molecule yields three products, one coincident with added 3H-labelled m5 rRNA in electrophoretic mobility, a second about forty nucleotides in length (designated F1) and a third composed of 22 nucleotides (designated F2). Two-dimensional electrophoretic analysis (data not shown) of T1 and pancreatic RNase digests of the 5S material generated during the reaction shows that it possesses the same 5' (p-U-U-U-G-) and 3' (-A-A-G- $C_{OH}$ ) termini as 5S rRNA obtained from ribosomes; the molecules are identical. The products of p5<sub>B</sub> cleavage include one component identical in size and structure to the added 3H-m5 rRNA and a second fragment having the same electrophoretic mobility and, as it proved, the same detailed structure as F2. Also derived from p5<sub>B</sub> is a component which is heterogeneous in size (about 8-15 nucleotides) which we have not characterised in detail.

Evaluation of the T1 RNase digestion products of the fragments released during the reaction defines their origin in the precursors (Fig. 2). Of particular note is that F1 contains -U-U-U-U-U-G<sub>OH</sub> (Fig. 2a), the 3' terminus of F1 and of p5<sub>A</sub> (M. Rosenberg, unpublished), whereas F2 yields p-U-G-(Fig. 2b), the 5' terminus of both precursors. F1 therefore must

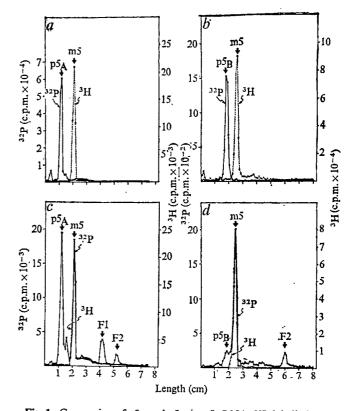


Fig. 1 Conversion of p5<sub>A</sub> and p5<sub>B</sub> to m5 rRNA. <sup>32</sup>P-labelled p5<sub>A</sub> or p5<sub>B</sub> prepared as described previously were incubated at 37° C for 15 min in the presence or absence of Sup 2 RNase M5 from B. subtilis 168 in 0.01 M Tris-HCl, pH 7.5, 0.05 M NH<sub>2</sub>Cl, 0.005 M MgCl<sub>2</sub>. Reaction was halted by addition of sodium dodecyl sulphate to 1% and ethylene diamine tetraacetic acid to 0.01 M, then 3H-labelled m5 rRNA was added as a size marker 0.01 M, then <sup>3</sup>H-labelled m5 rRNA was added as a size marker and products were resolved by electrophoresis through 8% polyacrylamide gels<sup>5</sup>. Materials migrating in gels between the m5 and F1 regions are products of nonspecific degradation. a, <sup>32</sup>P-p5<sub>A</sub> plus <sup>3</sup>H-m5 rRNA (no enzyme); b, <sup>32</sup>P-p5<sub>B</sub> plus <sup>3</sup>H-m5 rRNA (no enzyme); c, <sup>32</sup>P-p5<sub>B</sub> incubated with Sup 2 RNase M5 preparation; d, <sup>32</sup>P-p5<sub>B</sub> incubated with Sup 2 RNase M5 preparation. Sup 2 was prepared as follows: 2 gm of B. subtilis 168 cell paste was resuspended in 4 ml buffer SB (0.05 M Tris-HCl, pH 7.5, 0.06 M NH<sub>4</sub>Cl, 0.01 M MgCl<sub>2</sub>, 0.0001 M EDTA-Na<sub>2</sub>, 0.0001 M dithiothreitol, 5% glycerol) containing 10 μg ml<sup>-1</sup> DNase. Cells were disrupted with a French press, and the lysate was diluted twofold with buffer SB and cleared by centrifugation at 20,000 r.p.m. (Sorvall SS-34 rotor) for 20 min. The supernatant is Sup 0. Ribosomes were pelleted from Sup 0 by centrifugation at 40,000 r.p.m. somes were pelleted from Sup 0 by centrifugation at 40,000 r.p.m. (Spinco 40 rotor) for 2 h, resuspended in SB, adjusted to contain 0.2 M NH<sub>4</sub>Cl, and again pelleted. The supernatant is Sup 2.

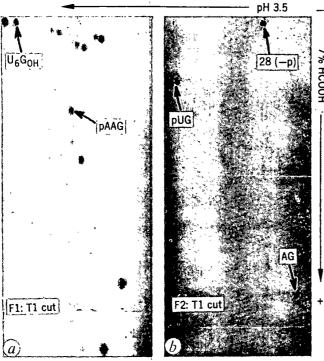


Fig. 2 Two-dimensional electrophoresis of T1 RNase digests of F1 and F2. Fragments designated F1 and F2 were generated from p5<sub>A</sub> by incubation with Sup 2 and resolved by gel electrophoresis as described in Fig. 1. Following elution from gels, the fragments were digested completely by T1 RNase and resulting oligonucleotides were resolved by two-dimensional electrophoresis on cellulose acetate at pH 3.5, followed by diethylaminoethyl paper at pH 1.9, as detailed by Sanger and Brownlee<sup>6</sup>, a, RNase T1 digest of in vitro F1; b, RNase T1 digest of in vitro F2.

represent the 3' and F2 the 5' terminal, precursor-specific segments of p5<sub>A</sub>. The scission events release 5'-phosphoryl and 3'-hydroxyl termini. Recovery of these fragments establishes that RNase M5 is a specific endonuclease.

We have not yet rigorously purified RNase M5 of *B. subtilis*, but substantial information regarding the enzyme is available (M.L.S., and N.R.P., unpublished). Apparently, a single catalytic activity is responsible for removing both the 5' and 3'

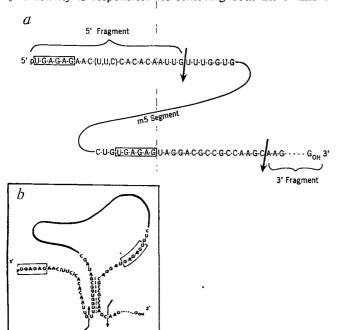
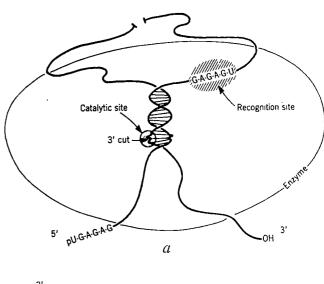


Fig. 3 Partial nucleotide sequence of p5<sub>A</sub> of B. subtilis; a, as a linear molecule and, b, with the 5' and 3' terminal regions of the m5 portion of the precursor juxtaposed in an antiparallel, duplex stalk.

terminal, precursor-specific segments of p5<sub>A</sub> and p5<sub>B</sub>. This is suggested by our inability to separate 5' and 3' cleavage activities by chromatography on DEAE cellulose or by zonal sedimentation through sucrose gradients. Moreover, the 5' and 3' cleavage activities display identical inactivation kinetics. RNase M5 is a large protein, 5-6S in size, corresponding to a molecular weight of 1 × 10<sup>5</sup>-1.5 × 10<sup>5</sup>. Maximal activity requires as well the presence in reactions of at least one relatively low molecular weight component, possibly a ribosomal protein. As a single enzyme removes both the 5' and 3' terminal precursor-specific sequences, it is likely that single catalytic and recognition sites are involved in interaction with the substrate molecules. In principle, therefore, it is possible to suggest features of the precursor molecules which interact with RNase M5 by identifying structural similarities in the vicinities of the two endonucleolytic cuts.

The nucleotide sequences of the precursor and mature 5S rRNA molecules from B. subtilis are almost completely determined (M.L.S. and N.R.P., and S. M. Weissman et al., unpublished). Relevant portions of the p5<sub>A</sub> molecule are shown in Fig. 3. The 5' and 3' terminal regions of the m5 portion of the precursor are juxtaposed in an antiparallel, duplex stalk. This feature is common to all 5S rRNA molecules so far examined and probably exists both in solution and in the ribosome. The points at which endonucleolytic cleavage occur are indicated. It is noteworthy that the cuts at the 5' and 3' termini of the molecule are spatially quite near to each other when the hydrogen-bonded stalk is constructed, and in fact the points of scission probably are within a duplex region. It seems unlikely, however,



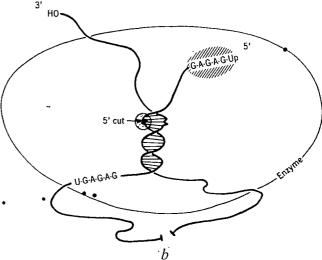


Fig. 4 Alternative models for the interaction of  $p5_A$  with RNase M5 (for an explanation of a and b see text).

that both endonucleolytic cuts are effected at the same time because the catalytic site on the enzyme surface very probably must consider the polarity of the phosphodiester bond being acted upon. The two bonds to be cleaved would present opposite polarities upon simultaneous inspection by a catalytic site.

It is evident from Fig. 3 that the sequences in the immediate vicinities of the endonucleolytic cuts are dissimilar; RNase M5 is unlikely to act at independent substrate sites on this basis. The sequence -U-G-A-G-A-G-, however, lies 16 nucleotides 5' removed from both points of cleavage. One of these sequences is at the 5' terminus of a precursor-specific segment whereas the second lies within the body of the m5 portion of the molecule. The probability of random assignment of two identical hexameric sequences equidistant from two given points (the cleaved sites) is about 2.4×10<sup>-4</sup>, so we suppose that this sequence constitutes at least part of that feature of the precursors which is recognised by the cleavage enzyme. If both the duplex stalk and the U-G-A-G-A-G sequence are considered to be involved in orientation of the substrate, then structural symmetry about the bonds to be cleaved is apparent, as is diagrammatically depicted in Fig. 4. Figure 4a positions the U-G-A-G-A-G- in the body of the m5 sequence at a 'recognition site' on the enzyme surface, and at the 'catalytic site' is placed the phosphodiester bond whose cleavage will release the 3' terminal, precursor-specific segment.

Alternately (or following release of the fragment) (Fig. 4b), the substrate could be inverted and rotated about the helical axis of the stalk to place the 5' terminal U-G-A-G-A-G into the 'recognition site'; this could place at the 'catalytic site' the phosphodiester bond whose hydrolysis releases the 5' terminal precursor-specific segment. Both U-G-A-G- sequences are aligned at the recognition site on the enzyme surface with the same chemical polarity and, similarly, the phosphodiester bonds to be cleaved are positioned at the catalytic site with the same polarity. The structural simplicity of the 5S rRNA molecule thus permits tentative identification of features within one type of polynucleotide which may interact in a highly specific fashion with a protein. The actual involvement of the proposed recognition-alignment elements and their relationships to the phosphodiester substrate bonds during the cleavage reaction are experimentally testable.

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### Candidate for immunoglobulin D present on murine B lymphocytes

ALTHOUGH the original discovery of human IgD is almost ten years old1, it is only very recently that a possible function for this class of immunoglobulin has been suggested. Present in normal human serum in very small amounts2, IgD is found on the surface membrane of relatively large numbers of human peripheral lymphocytes, in particular those obtained from cord blood and the circulation of patients with chronic lymphatic leukaemia3-7. IgD is frequently associated with IgM on the same cell<sup>5-7</sup> and this, together with the fact that the frequency of IgD-bearing lymphocytes is higher in cord blood than adult blood<sup>4,5</sup>, suggests a fundamental role for IgD, either as a primitive recognition unit or for regulation of the immune response. Were this to be so, then clearly IgD would be expected to occur in species other than man. Here we present the identification of an immunoglobulin present on the surface of mouse splenic lymphocytes which is not IgM, but which has the predicted characteristics of IgD.

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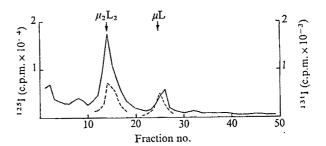


Fig. 1 Surface immunoglobulin of murine splenic lymphocytes. Spleen cell suspensions of 4-6-week-old female CBA mice were labelled with <sup>125</sup>I by the lactoperoxidase catalysed procedure<sup>8</sup>, washed once in phosphate-buffered saline (PBS) and then lysed for 10 min at 0°C in 1% (w/v) Nonidet P40 in PBS. The centrifuged lysate (4,000g×10 min), containing 95% of the total TCA-precipitable radioactivity, was dialysed against 1% (w/v) Nonidet P40-PBS in the cold. Surface-labelled immunoglobulin was precipitated by the addition of a polyspecific rabbit antimouse Ig serum (10 µl) followed by goat anti-rabbit IgG (100 µl). The precipitate was washed three times with ice cold 0.5% (w/v) Nonidet P40 in PBS, twice with ice cold PBS, and then dissolved Nonidet P40 in PBS, twice with ice cold PBS, and then dissolved in 4% (w/v) sodium dodecyl sulphate (0.1 M iodoacetamide) 0.05 sodium phosphate, pH 7.0, by heating at 100° C for 5 min. A sample of mouse myeloma MOPC 104E IgM was labelled with <sup>131</sup>I (ref. 16), partially reduced to  $\mu_2 L_2$  and  $\mu L$  by treatment with 1 mM dithiothreitol for 60 min at room temperature, alkylated with 10 mM iodoacetamide, and then added to the isolated <sup>125</sup>I-cell surface Ig to serve as an internal marker for the gel analysis (4.25% (w/v) polyacrylamide gel containing for the gel analysis (4.25% (w/v)) polyacrylamide gel containing 0.1% (w/v) sodium dodecyl sulphate). Following electrophoresis, the gel was sliced into 1 mm segments and radioactivity determined. Values were corrected for cross channel spill, and plotted with the top of the gel on the left hand side of the figure.

—, <sup>125</sup>I incorporated into cell surface lg; ---, <sup>131</sup>I-labelled internal marker.

Spleen cell suspensions containing more than 95% viable cells (trypan blue exclusion) were labelled with 125 by the lactoperoxidase-catalysed procedure8, and the surface immunoglobulin was prepared and analysed on sodium dodecyl sulphate (SDS) gels9 with addition of an internal marker (131I-labelled IgM<sup>10</sup> partially reduced to  $\mu_2L_2$  and  $\mu L$ ). As has been previously described11,12, we found a major portion of radioactive cell surface immunoglobulin in that part of the gel corresponding to the IgMs (µ2L2) subunit of IgM (Fig. 1). In addition, however, there was a significant radioactive peak running very slightly in advance of the µL subunit, but which contained heavy and light chains on reduction (see Fig. 2) and must therefore be an HL subunit.

Following reduction and alkylation both the H<sub>2</sub>L<sub>2</sub> and HL subunits were shown to contain two distinct species of heavy chain, there being one component with the mobility (and therefore size) of the internal 131I-µ chain marker, and another which migrated faster than  $\mu$  chain, but slower than  $\gamma$  chain (Fig. 2). The proportion of radioactivity found in the smaller (faster migrating) heavy chain was lower in the sample of reduced <sup>125</sup>I-surface H<sub>2</sub>L<sub>2</sub> (Fig. 2a) than the reduced <sup>125</sup>I-surface HL (Fig. 2b).

We discount the possibility that the heterogeneity of surface

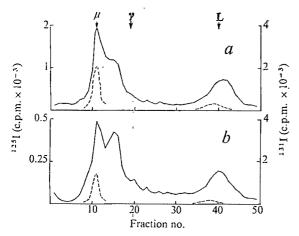


Fig. 2 Heavy chain heterogeneity of surface immunoglobulin of murine splenic lymphocytes. Sections of the gel shown in Fig. 1 containing the  $H_2L_2$  and HL components were eluted with 2% (w/v) sodium dodecyl sulphate (2mM dithiothreitol) 0.05 M sodium phosphate, pH 7.0. The eluate was heated for 15 min at  $100^{\circ}$  C in order to ensure reduction of all disulphide bridges, and then alkylated by addition of iodoacetamide to 10 mM. The resulting material was then applied to 10% (w/v) polyacrylamide gels containing sodium dodecyl sulphate to resolve the heavy chain components. In these conditions the  $^{131}I_{-}\mu_2L_2$  and  $\mu$ L originally added as internal markers for the first gel are also extracted and reduced, thereby providing internal markers for  $\mu$  and L chains in the second separation in the 10% acrylamide gels. The gels were fractionated, counted and plotted as given in the legend to Fig. 2. The position of  $\gamma$  chains was assessed by running a mixture of totally reduced  $^{131}I_1$ -labelled mouse IgM (MOPC 104E) and IgG2a (Adj.PC5) on a parallel gel. a, Reduced, surface labelled  $H_2L_2$ ; b, reduced, surface labelled  $H_L$ . ——,  $^{125}I_1$  incorporated into cell-surface Ig; ——,  $^{131}I_2$ -labelled internal marker.

Ig results from the presence of T-lymphocytes in the spleen cell suspension as similar results were obtained when spleen cells from nude mice were analysed by the same techniques. In addition, peripheral T-lymphocytes purified by passage of spleen cells through nylon wool columns<sup>13</sup> did not contain detectable amounts of surface immunoglobulin using the methods described above.

Reconstruction experiments were performed to check for degradation of  $\mu$  chain-containing material during the preparation of labelled surface Ig. Both  $^{126}\text{I-labelled}~\mu_2L_2$  and  $\mu\text{L},$  prepared by partial reduction of MOPC 104E IgM, could be recovered unchanged when added to spleen cells and then carried through the procedures outlined above for isolation of cell surface immunoglobulin. The HL subunits found on the surface of splenic lymphocytes, therefore, do not result from reductive depolymerisation of  $\mu_2L_2$  during isolation. Reduction of the recovered  $^{125}\text{I-}\mu_2L_2$  and  $^{126}\text{I-}\mu\text{L}$  yielded heavy chains entirely corresponding in size to untreated  $\mu$  chain. We are therefore confident that the observed heavy chain size heterogeneity of cell surface immunoglobulin does not result from degradation of  $\mu$  chain during the isolation procedure.

A possible explanation for the heterogeneity of surface immunoglobulin is that there is a precursor-product relationship between the various species observed. Such a relationship could either reflect a biosynthetic pathway or metabolic turnover of membrane-associated immunoglobulin. In the first case the HL subunit would be an intermediate in the biosynthesis of H<sub>2</sub>L<sub>2</sub>, and the small heavy chain would be a partially glycosylated  $\mu$  chain requiring the addition of further sugar residues for completion. In the alternative situation, the direction would be expected to be towards degradation of H<sub>2</sub>L<sub>2</sub> to HL and proteolysis of the heavy chain. We have ruled out either possibility by culturing surface-labelled spleen cells in vitro for 0-12 h. The absence of any form of precursorproduct relationship was clearly indicated by the fact that the ratios of H<sub>2</sub>L<sub>2</sub> to HL and large heavy chain (that is, a μ chain) to small heavy chain determined at time zero remained the

same in cell surface and released immunoglobulin recovered at all times of incubation. This experiment demonstrated also that the half lives of all the immunoglobulin species present on the cell surface are similar, a value of 10 h being determined.

We then pursued the possibility that the small heavy chain was derived from a class other than IgM by using antisera of defined class specificity for isolation of surface immunoglobulin. Antibodies specific for  $\gamma 1$ ,  $\gamma 2a$  and  $\alpha$  chains did not precipitate immunoglobulin from detergent-solubilised, surface-labelled splenic lymphocytes. On the other hand, specific anti-µ chainprecipitated H2L2 and HL containing heavy chain corresponding only in size to authentic, secreted  $\mu$  chain (Fig. 3a). This material may therefore be identified as  $\mu$  chain with certainty. When the supernatant from the anti-µ precipitation was further reacted with polyspecific anti-(mouse Ig), however, the H<sub>2</sub>L<sub>2</sub> and H1 species precipitated were predominantly composed of the small heavy chain (Fig. 3b). We may therefore conclude that there is present on the surface of murine splenic lymphocytes a heavy chain larger than the  $\gamma$  chain, yet smaller than the  $\mu$  chain, which is not precipitated with antisera to  $\gamma$ , α or μ chains, but which is reactive with a polyspecific antiserum to mouse Ig. As the small heavy chain was not precipitated by anti-(µ chain), the possibility that it is an incompletely glycosylated µ chain<sup>14</sup> is ruled out.

The inescapable conclusion is that this heavy chain, which accounts for about 40% of heavy chain isolated from surface-labelled spleen cells, represents a hitherto undescribed class of

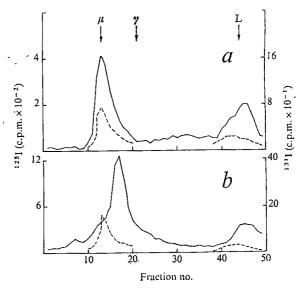


Fig. 3 Two different heavy chain classes present on the surface of murine splenic lymphocytes. Spleen cells were labelled with <sup>125</sup>I as described in the legend to Fig. 1. They were washed once in Eagle's medium containing 10% foetal calf serum, resuspended in the same medium at 2×10<sup>7</sup> cells ml<sup>-1</sup>, and then incubated at 37° C in an atmosphere of 5% CO<sub>2</sub> in air for 6 h. At the end of the incubation period, the cells were 95% viable (trypan blue exclusion). The medium, containing released cell surface immunoglobulin, was separated from the cells by centrifugation (400g, 10 min), adjusted to contain 1% (w/v) Nonidet P40, and dialysed against 1% (w/v) Nonidet P40 in phosphate buffered saline. To the non-diffusate was added a specific rabbit anti-(mouse μ chain) serum (10 μl) and goat anti-(rabbit IgG) serum (100 μl). The resulting precipitate was removed by centrifugation, and the supernatant was further reacted with polyspecific rabbit anti-(mouse Ig (40 μg) to ensure complete precipitation of mouse Ig. Both precipitates were washed and analysed in 4.2% (w/v) polyacrylamide gels as described in the legend to Fig. 1. The H<sub>2</sub>L<sub>2</sub> containing regions of both gels were eluted, reduced and alkylated, and analysed on 10% (w/v) polyacrylamide gels as described in the legend to Fig. 2. a, Reduced cell surface H<sub>2</sub>L<sub>2</sub> precipitated with rabbit anti-(mouse μ chain); b, reduced cell surface H<sub>3</sub>L<sub>2</sub> prepared from the supernatant of the precipitation with rabbit anti-(mouse μ chain) by addition of rabbit anti-(mouse Ig).

—, <sup>125</sup>I incorporated into cell-surface Ig; ---, <sup>131</sup>I-labelled internal marker.

immunoglobulin in the mouse; the most obvious surmise is that it corresponds to the human IgD class. To support this suggestion we note the similar mobility on SDS-polyacrylamide gels of human δ chain<sup>2</sup> and the novel heavy chain of mouse lymphocytes described above. This newly described mouse immunoglobulin, which we shall now refer to as IgD, is also similar to human IgD2 in its marked susceptibility to proteolytic degradation (E.R.A. and R.M.E.P., unpublished). A similar conclusion has also been drawn by others (E. S. Vitetta and J. W. Uhr, unpublished).

Contrary to our expectation, however, was the finding of  $\mu$  chain, but not  $\delta$  chain, in foetal liver (16 d embryos) and neonatal spleen and liver. In addition, splenic lymphocytes of 6 week and 6 month old mice contained similar amounts of  $\delta$  chain, the ratio of  $\mu$ : $\delta$  being about 3:2. This could mean that the expression of IgD in the mouse is subsequent to the appearance of IgM. If this is not the case, then IgD-bearing lymphocytes must arise in organs other than spleen or foetal liver. Perhaps relevant to this point is the fact that IgD constitutes the major immunoglobulin class present on murine lymph node cells, where the  $\mu$ : $\delta$  ratio was found to be 1:3.5-4.0.

It is certainly intriguing that spleen and lymph node cells differ markedly in terms of the relative amounts of  $\mu$  and  $\delta$ chains present on their surfaces. In this respect we may note that spleen cells, but not lymph node cells, respond to lipopolysaccharide (G. Janossy and R.M.E.P., unpublished), and that spleen cells secrete largely IgM, whereas the major product of lymph node cells is IgG15. The presence or absence, however, of a functional relationship between these separate observations remains to be determined. Whether the molecular heterogeneity of total cell surface Ig is reflected in a similar pattern on individual cells is not known. Nonetheless, a relationship between this observed heterogeneity and regulation of the B-lymphocyte response to antigen is suggested.

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#### Immunoglobulin of T lymphoma cells is an integral membrane protein

IMMUNOGLOBULINS, predominantly 7S IgM molecules (molecular weight ~ 200,000), are associated with the plasma membranes of lymphocytes1-3 and considerable evidence suggests that these proteins function as receptors for antigen<sup>4-6</sup>. If these molecules are themselves integral parts of the lymphocyte membrane or are strongly associated with integral proteins they may transmit antigen-induced signals through the membrane and initiate cell activation. It has been proposed that the antigen-combining site of T cell IgM protrudes from the cell surface, but that the Fc portion bearing the characteristic antigenic determinants of the heavy chain is not accessible to large molecules in the solvent<sup>2,7</sup>. If these proposals are correct, then surface immunoglobulin of T cells might be considered an integral constituent of the plasma membrane8.

Cultured mouse WEHI-22 T lymphoma cells represent monoclonal T cells which synthesise and express surface IgM-like immunoglobulin<sup>2,5,9</sup> showing certain collaborative properties<sup>10</sup>. If immunoglobulin is an integral protein of WEHI-22 plasma membrane, it should be possible to isolate the plasma membrane and to detect immunoglobulin associated with it. Because preparation of purified membranes involves large dilution factors and prolonged washing<sup>11</sup>, it is likely that loosely associated peripheral proteins would be lost during the process. In this communication we provide evidence that immunoglobulin is detectable on purified plasma membrane of WEHI-22 cells and is therefore strongly associated with the T cell membrane.

Plasma membranes were prepared from unlabelled WEHI-22 cells to which were added 5×107 surface radioiodinated WEHI-22 cells. The cells were radioiodinated by a modification<sup>12</sup> of the lactoperoxidase method developed for labelling

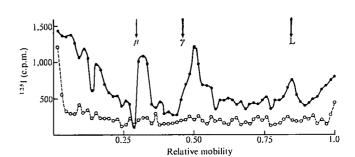


Fig. 1 Polyacrylamide gel electrophoresis in SDS of 125 I-labelled polypeptide chains of immunoglobulin isolated from membranes of T lymphoma cells of the line WEHI-22. Membrane protein (225 µg) was solubilised in 500 µl 1% Nonidet P40-6M urea. The solution was dialysed against PBS and subsequently the nondialysable protein was radioiodinated using chloramine-T (ref. 15): to 100 µl membrane protein solution (0.45 mg protein ml<sup>-1</sup>) were added 10 µl carrier-free <sup>125</sup>I (100 mCi ml<sup>-2</sup>) and 5 µl chloramine-T (1 mg ml<sup>-1</sup>) and then the mixture was held at 4° C for 1 h. The reaction mixture was fractionated by gel filtration on Sephadex G100 in 0.5% Nonidet P40 in PBS. Fractions of the <sup>125</sup>I-labelled high molecular weight peak were pooled and PBS added to a total volume of 3.0 ml. Aliquots (200 µl) of this 125I-labelled membrane protein solution were precipitated with the specific precipitation system which consisted of mouse IgM (100 µl of a concentration of 0.1 mg ml<sup>-1</sup>) and rabbit antiserum to mouse IgM (100 µl of antiserum diluted 1 in 2) or with the control precipitation system which consisted of fowl IgG (100 µl of a concentration of 0.1 mg ml<sup>-1</sup>) and rabbit antiserum to fowl IgG (100 µl of antiserum diluted 1 in 2). The coprecipitates were washed three times with PBS, dissolved in a buffer containing 10% glycerol, 5% mercaptoethanol, 3% SDS in 0.125 M Tris-HCl, pH 6.8 which was 6M in urea and then analysed by polyacrylamide gel electrophoresis (10% acrylamide, 0.25% bisacrylamide) in a discontinuous buffer system according to the method of Lagranghia Specific precipitates: method of Laemmli<sup>18</sup>.  $\bullet$ , Specific precipitates;  $\bigcirc$ , control precipitates;  $\mu$ ,  $\gamma$  and L refer to positions of standard immunoglobulin chains.

lymphocytes<sup>13</sup>. The label was used solely to monitor the distribution of plasma membrane during fractionation and the subsequent analysis of the membrane proteins (Fig. 2). The cells were disrupted using a modification of the "pump" described by Wright et al.14 and the plasma membrane was separated according to the method of Crumpton and Snary<sup>11</sup>. The purified membrane which exhibited a 35-fold enrichment in specific 5'-nucleotidase activity relative to the initial cell homogenate was solubilised in 1% Nonidet P40-6M urea. Approximately 95% of the radioactivity was obtained in solution. This solution was dialysed against PBS and the nondialysable protein radioiodinated using the chloramine-T method (ref. 15 and legend to Fig. 1). The reaction mixture was fractionated by gel filtration on Sephadex G-100. Approximately 70% of the added 125I-iodide was associated with high molecular weight protein as judged by gel filtration.

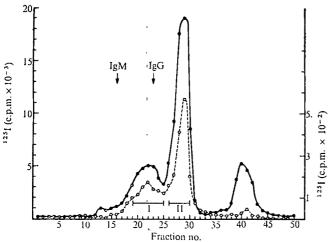


Fig. 2 Gel filtration on Sepharose 6B of solubilised membranes of T lymphoma cells of the line WEHI-22. Two procedures were used: a, membranes were solubilised in 1% sodium deoxycholate (Merck) in 20 mM Tris-HCl, pH 8.2 (0.5 mg membrane protein per ml solvent; 1 h at room temperature) and the Sepharose 6B column (55 cm $\times$ 0.8 cm) was developed in the same solvent ( $\bullet$ ). b, Membranes were solubilised in 10M urea-1.5 M acetic acid (0.5 ml membrane protein per ml solvent; 2 h at 30° C) followed by dialysis against Tris-NaCl, pH 8.0; 15% of the original membrane bound counts were soluble in Tris-NaCl as judged by centrifugation. Separation was performed on a Sepharose 6B column of identical dimensions, the elution buffer was PBS (O). The fractions indicated I and II obtained from the separation using procedure B were pooled, concentrated by ultrafiltration and radioiodinated using chloramine-T<sup>15</sup>. These fractions were assessed for the presence of Ig by coprecipitation (legend to Fig. 1). IgM and IgG represent the position of intact marker proteins (molecular weights 900,000 and 150,000, respectively) resolved under these conditions

The presence of Ig in the solubilised 125I-labelled membrane was ascertained by immunological precipitation. We found that the specific immune precipitate possessed  $7.7\pm0.5\times10^3$ c.p.m. whereas the control precipitate gave  $1.1\pm0.1\times10^3$ c.p.m. This difference shows that the plasma membrane contains Ig. This interpretation is supported by polyacrylamide gel electrophoretic analysis of the immune precipitates in buffers containing sodium dodecylsulphate (SDS)16. Furthermore, the results of these analyses closely resembled those reported elsewhere for WEHI-22 surface Ig isolated from lactoperoxidase-iodinated whole cells (ref. 2 and D.H., J.J.M. and A. W. Harris, submitted for publication). Radioiodinated plasma membrane Ig contained components resembling μ-chain and L-chain in their electrophoretic mobilities (Fig. 1). A third component was also present with a mobility consistent with a molecular weight of about 40,000. Studies to be reported in detail elsewhere revealed that this component has properties similar to those predicted for the surface membrane receptor for the Fc-portion of exogenous IgG (ref. 17). A considerable amount of material with mobilities greater than that corresponding to a molecular weight of 105 was observed in all

polyacrylamide gels of homologous immune precipitates derived from preparations of plasma membrane radioiodinated using chloramine-T. The amount and distribution of this material was, however, variable and studies of its nature were not pursued further; similar material was observed in Ig isolated from murine B cells radioiodinated using chloramine-T (E. White, and J.J.M., unpublished observations).

These results establish that light chains and μ-like heavy chains were present in surface membrane Ig of WEHI-22 cells; but, they do not provide any information regarding the intact state of the molecule. This problem has been approached by fractionating solubilised membrane by gel filtration on Sepharose 6B (legend to Fig. 1) and radioiodinating the fractions eluted in positions corresponding to molecular weights of  $<10^5$  and between  $1\times10^5$  and  $3\times10^5$  in order to ascertain the presence or absence of free polypeptide chains and 7S Ig respectively. These fractions are illustrated in Fig. 2. The fractions termed I and II were pooled as indicated and radioiodinated using chloramine-T (ref. 15). Immunoglobulin estimated by immune precipitation, was found only in the 100,000-300,000 molecular weight fraction  $(3.6\pm0.6\times10^3$ c.p.m. specific;  $1.1\pm0.1\times10^3$  c.p.m. control). No Ig was detected in the lower molecular weight fraction  $(0.9 \pm 0.2 \times 10^3)$ c.p.m. specific;  $1.1\pm0.2\times10^3$  c.p.m. control). There was insufficient material in the elution position of 19S IgM to enable analysis of this fraction. These results are consistent with the previous observation that the Ig associated with the surface of T cells exists primarily as 7S units2,5 rather than as free polypeptide chains18.

Surface Ig of mouse T lymphoma cells of the line WEHI-22 was found to exist as a 7S IgM unit which was bound to the plasma membrane sufficiently strongly to resist dissociation or elution during the disruption of the cells and the isolation of the plasma membrane. These results are consistent with the proposals that either T cell immunoglobulin is itself an integral membrane protein or that it is tightly associated with another component buried in the lipid bilayer of the membrane. Similar proposals also apply to the surface IgM of the human lymphoblastoid cell line BR18 (M. J. Hayman, and M.J.C., unpublished observations).

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then rises steadily to reach a plateau by about day 5 although the cells continue in log phase growth for a further 5-6 d.

Inhibition of agglutinating activity was attempted with monospecific antisera directed against each of the major classes of human Ig heavy and light chains, in experiments of the type shown in Fig. 1. The results with three of the lines were clear cut (Table 1), indicating that the activity is associated with Ig of class IgM\(\lambda\) in the supernatants of HIL1, ANA1 and PEN2. In the case of BRIs the agglutinating titre of the supernatant is low and the findings less easy to interpret. Anti-k serum consistently inhibits agglutination, but both anti-A and anti-M (and occasionally anti-G) also seem to have some inhibitory effect. Attempts to identify Igs by immunoelectrophoresis of concentrated supernatants from cell lines ANA1, PEN2 and BRI8 were only partially successful. The presence of  $\lambda$  light chains in material from ANA1 and PEN2 was confirmed but no other heavy or light chains could be demonstrated. Failure to detect Igs, when their initial concentration in culture supernatant is low, is common with this technique<sup>2</sup>.

#### Antibody activity associated with immunoglobulins synthesised by human lymphoblastoid cell lines

Antibody activity has not previously been detected in association with immunoglobulins synthesised spontaneously by human lymphoblastoid cell lines in vitro. One reviewer has commented "if some antibody activity was to be found, the established cell lines would be a fascinating tool for the study of homogenous antibodies". We here report such a finding.

In the course of studies on immunoglobulin (Ig) production in vitro, by means of a haemagglutination-inhibition assay2, supernatants from four human lymphoblastoid lines (of more than 60 tested) were found to agglutinate glutaraldehyde-fixed Ig-coated sheep erythrocytes in the absence of anti-Ig serum. Details of the four lines are given in Table 1. It was established that the Ig coating is irrelevant to the agglutination reaction and that supernatants from these four lines (but not from others) will agglutinate sheep or human erythrocytes fixed with glutaraldehyde or formaldehyde. There is no detectable activity against fresh erythrocytes of either species, nor against glutaraldehyde or formalin-fixed calf erythrocytes. Tanned or glutaraldehyde-fixed horse erythrocytes agglutinate spontaneously in the presence of unused culture medium (Ham's F10 with 10% tryptose phosphate broth and 20% foetal calf serum). The agglutinating activity against glutaraldehyde-fixed sheep red cells seems to be independent of temperature over the range 4° C to 37° C.

The kinetics of agglutinin production have been studied in cell line PEN<sub>2</sub>. Some activity is detectable within 24 h of resuspending the cells at 105 ml<sup>-1</sup> in fresh medium. The titre Dilutions of antiserum

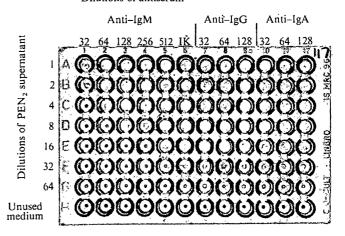


Fig. 1 Haemagglutination carried out in round-bottomed wells of microtest II plate (Linbro plastics). Reciprocal dilutions of PEN<sub>2</sub> supernatant (in fresh culture medium containing 20 foetal calf serum) are recorded on the left; reciprocal dilutions of monospecific anti-human Ig chain sera (in physiological saline) are recorded above. One drop of diluted supernatant was mixed with 1 drop of diluted antiserum in each well and 30 min later 1 drop of a 1 % suspension (in saline) of sheep red cells, previously fixed in 2.5% glutaraldehyde, was added. The cells were allowed to settle overnight at room temperature. Agglutination is shown by the absence of a dark central pellet of erythrocytes. Inhibition of the agglutination reaction becomes more marked with increasing concentrations of anti-IgM serum. There is no inhibition demonstrable with anti-IgG or anti-IgA sera.

		Table 1	Cell lines p	producing a	gglutinin aga	inst fixed	i sheep o	r human	erythrocy	/tes		
	Donor Shell cell agglutinin in supernatant											
Cell line ANA <sub>1</sub>	Sex M	Diagnosis Healthy infant	La	boratory of origin* MRC	Maximum titre† 1:32	Anti-G	Anti-A	In Anti-M +	hibited b Anti-K —		GPK —	OxRBC +
$HIL_1$	F	Healthy infant		MRC	1:32	_	****	+		+	Section 1	+
PEN <sub>2</sub>	M	Klinefelter's syndrom (47,XXY)		MRC	1:32	-		+	***	+	****	-
$BRI_8$	M	Healthy adult		Searle	1:8	士	+	+	+	_	***	-

\* MRC is the authors' laboratory; Searle, G. D. Searle and Company, Research Division, High Wycombe, UK.
† Maximum dilution of supernatant giving positive result against 1% suspension of glutaraldehyde-fixed sheep erythrocytes. Titres against human cells or against formalin-fixed sheep erythrocytes are usually lower. Recently, using an Q.25 % suspension of glutaraldehyde-fixed sheep

erythrocytes, titres of up to 1:256 have been observed in PEN<sub>2</sub> supernatants.

‡ Anti-G, Anti-A and Anti-M, Monospecific goat anti-human heavy chain sera (Technicon); Anti-K and Anti-L, monospecific rabbit antihuman light chain sera (Hoechst); GPK, OxRBC, guinea pig kidney and ox erythrocyte stroma extracts for infectious mononucleosis ('Monospot') tests (Ortho diagnostics).

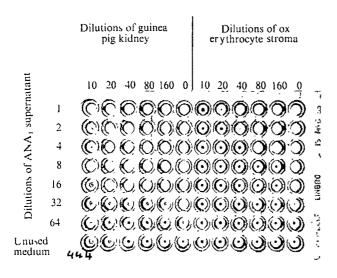


Fig. 2 Haemagglutination test set up as in Fig. 1. Reciprocal dilutions of ANA, supernatant are recorded on the left. One drop of the diluted supernatant was mixed with one drop of guinea pig kidney or ox erythrocyte stroma extracts (Ortho Diagnostics) in saline (reciprocal dilutions shown above) in each well of the test plate. One drop of glutaraldehyde-fixed sheep erythrocytes (1% suspension in saline) was added 20 min later and the plate left overnight at room temperature. Progressive inhibition of agglutination with increasing concentrations of ox erythrocyte stroma is clearly demonstrated. Guinea pig kidney extract has no apparent effect on the agglutination reaction.

Preliminary studies on the physical properties of the erythrocyte-agglutinating factor from PEN2 supernatants show that it is precipitated by 10-40% saturated ammonium sulphate. It can be isolated as a single sharp peak from a Sephadex G200 column and the major fraction bands with a human IgM marker on a 10-40% sucrose gradient. There is a minor peak of agglutinating activity in the lower molecular weight range, possibly representing depolymerised IgM.

There seems to be no common factor in the lines or in their origins which distinguishes these four cultures from others established and maintained in identical conditions. The four lines are readily distinguished from each other by cytogenetic and isoenzyme analysis. A second line, ANA2, was established from the same blood sample which gave rise to ANA1. No erythrocyte agglutinating activity is detectable in its supernatant.

The original sera from the donors of PEN<sub>2</sub>, ANA<sub>1</sub> and HIL<sub>1</sub> were recovered from storage at  $-20^{\circ}$  C along with samples from 20 other donors including three cord bloods and nine from patients with Klinefelter's Syndrome (karyotype 47,XXY or 49,XXXXY). Lymphoblastoid cell lines had been established from most of these control donors. The sera were tested for agglutinating activity against glutaraldehyde-fixed sheep red cells. More than half (including those from the donors of PEN<sub>2</sub> and HIL<sub>1</sub>) gave weak reactions at dilutions of 1:5 or 1:10. These were considered non-specific. Four Paul-Bunnel positive samples from patients with infectious mononucleosis agglutinated the fixed sheep cells at titres of 1:160 to >1:640. As in the Paul-Bunnel reaction, this agglutination was inhibited by adsorption with ox erythrocyte stroma but not with guinea pig kidney extract3. Similar tests on the four cell line supernatants show that those from ANA<sub>1</sub> and HIL<sub>1</sub> consistently behave like infectious mononucleosis sera (Fig. 2), but agglutinating activity in PEN2 or BRI8 supernatants seems not to be inhibited by ox erythrocyte or by guinea pig kidney extracts.

These preliminary data suggest that EB virus, which is implicated both in infectious mononucleosis4 and in the proliferation of human lymphoblastoid cell lines in vitro<sup>5,6</sup>, may be responsible for the production of agglutinins directed against fixed sheep erythrocytes by at least some of these lines.

Further work is in progress to isolate and compare the agglutinins synthesised by the four cell lines under study.

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#### Tolerance induction with bovine y globulin in mouse radiation chimaeras depends on macrophages

THE injection of adult mice with ultracentrifuged heterologous y globulin induces immunological unresponsiveness to a subsequent injection of the globulin incorporated in incomplete or complete Freund's adjuvant1. It has been reported that inbred strains of mice differ in their susceptibility to the induction of unresponsiveness. Bovine y globulin (BGG), human γ globulin (HGG) and rabbit γ globulin (RGG) easily produce tolerance in C57BL/6 and DBA/2 mice but not in BALB/c mice<sup>2,3</sup>. Passage of BGG through the circulation of BALB/c mice (but not DBA/2 mice) gives preparations which produce a high degree of tolerance in the recipient mice suggesting that strain differences in the induction of tolerance are related to the capacity of the reticuloendothelial system to process the immunogenic component in ultracentrifuged soluble BGG (sBGG). Here, we report that the administration of carrageenin, which is known to be toxic for macrophages destroys the resistance to tolerance induction in BALB/c mice. Also spleen and bone marrow cells transferred from BALB/c mice to irradiated DBA/2 mice to form chimaeras, do not show characteristic BALB/c resistance to induction of tolerance until twelve weeks after transfer.

To test immune responsiveness to BGG we used previously described procedures<sup>2,3</sup>. One week after intraperitoneal (i.p.) injection of 2.0 mg sBGG for tolerance induction the mice

Table 1 Effect of various doses of carrageenin on the induction of unresponsiveness with 2 mg in BALB/c mice

Carrageenin treatment		ne respons	iveness
		Partially tolerant	
0.5 mg 3 d and 1 d before sBGG 4×0.5 mg every second day before	2	3	4
sBGG. • • • 4×0.5 mg every second day before	11	6	2
saline None			5 5

Kappa carrageenin (Marine Colloids) was dissolved in saline by heating, and an appropriate dose administered in 0.5 ml., 2.0 mg sBGG was administered 24 h after the last injection of carrageenin. One week later the mice were immunised with 100 µg BGG-CFA and then challenged with 2 µg 126I-BGG. were immunised with 100 μg BGG in complete Freund's adjuvant (CFA). Five days later they were given 0.1% KI in their drinking water. One week after immunisation the mice were challenged with 2 μg <sup>125</sup>I-BGG and whole body counts obtained daily in a gamma scintillation counter. Two milligrams sBGG readily induces tolerance in DBA/2 mice. BALB/c mice exhibit complete resistance to the induction of unresponsiveness at this dose and show subsequent clearance rates of <sup>125</sup>I-BGG indistinguishable from the immune control animals.

Carrageenin, a polysaccharide isolated from seaweed, known to be toxic for macrophages<sup>4</sup> but not for lymphocytes<sup>5,6</sup> was tested for its effect on tolerance induction by administration to BALB/c mice, before the injection of sBGG. Pretreatment with carrageenin favours tolerance induction in BALB/c mice (groups 1 and 2) whereas the immune response of the mice treated with BGG-CFA only (group 3) was not affected (Table 1). Thus, carrageenin-sensitive cells seem to be a critical factor in susceptibility to the induction of unresponsiveness.

We studied further this difference using cell transfer experiments. DBA/2 mice share the major histocompatibility loci (H-2<sup>d</sup>) with BALB/c mice and are poor responders to most bone marrow allografts<sup>7,8</sup>. Previous spleen cell transfer experiments showed that although the animals were true chimaeras with respect to their content of donor T and B cells at 4 weeks after lethal irradiation and reconstitution, they responded to the induction of tolerance to sBGG like normal animals of recipient strains<sup>9</sup>. We wished to examine the change with time of the susceptibility to the induction of unresponsiveness in lethally irradiated (850 rad) DBA/2 mice reconstituted with 2 × 10<sup>7</sup> BALB/c bone marrow cells from 8-week-old donors.

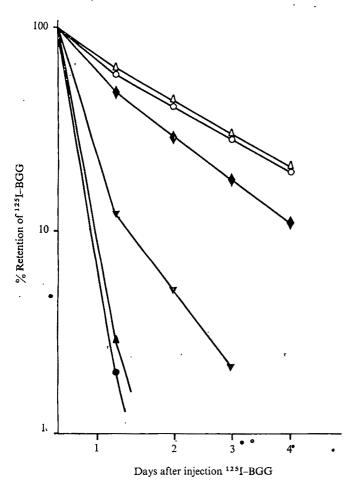


Fig. 1 Induction of tolerance in DBA/2 mice 12 weeks after the reconstitution with BALB/c bone marrow cells. Clearance rate 2 μg <sup>125</sup>I-BGG in normal (N) and irradiated-reconstituted (I-R) animals: Δ, N, saline-CFA; ⊙, I-R, saline-CFA; ♠, N, 2 mg sBGG+BGG-CFA; ▼ I-R, 2 mg sBGG+BGG-CFA; ▲ N, BGG-CFA; ♠ I-R, BGG-CFA.

This selected host-donor combination displayed practically no secondary disease and low mortality (5 out of 45 animals, in 4 months after irradiation).

As in previous spleen cell transfer experiments, animals tested for tolerance induction 4 weeks after reconstitution with BALB/c bone marrow cells showed non-immune clearance characteristic of normal DBA/2 mice made tolerant (Table 2). Six weeks after reconstitution the results were similar. But after 12 weeks 10 out of 11 animals exhibited the characteristic resistance to tolerance of the BALB/c donors. At that time 2 mg sBGG failed to induce tolerance in DBA/2 animals reconstituted with BALB/c cells although the same dose induced tolerance in the non-irradiated controls (Fig. 1). No significant difference was observed in the ability of BALB/c marrow-reconstituted and normal DBA/2 mice to clear 2 µg 125I-BGG when pretreated with CFA only, or to respond to an immunising dose of BGG-CFA (Fig. 1).

Table 2 Induction of unresponsiveness to sBGG in irradiated DBA/2 mice reconstituted with BALB/c bone marrow cells

Immune responsiveness	Weeks after reconstitution				
	4	6	12		
Tolerant	8	5	0		
Partially tolerant	0	1	1		
Immune	0	0	10		

Mice are irradiated with 850 rad and reconstituted with  $2\times10^7$  bone marrow cells. They were injected with 2 mg sBGG 4, 6, or 12 weeks after reconstitution. One week later the mice were immunised with 100  $\mu$ g BGG-CFA and then challenged with 2  $\mu$ g <sup>125</sup>I-BGG.

These results demonstrate that the resistance of BALB/c mice to tolerance induction resides in a cell population sensitive to the action of carrageenin. The most likely candidate would be macrophages in the reticuloendothelial system which are responsible for effectively processing the immunogenic portion of BGG and initiating a response in some clones before tolerance is induced in the remainder. On subsequent immunisation these primed cells proceed to make antibody and the mice seem on balance to be immune. The literature is full of observations demonstrating an enhanced immune response following stimulation of macrophages and suppression following their destruction<sup>10</sup>.

What is somewhat surprising is the failure of spleen and bone marrow cell grafts to effect reconstitution of an irradiated recipient in the sense that they would then behave like donors in respect to tolerance induction. Balner<sup>11</sup> however, has shown that after irradiation and bone marrow grafting, host type macrophages in the peritoneum were not replaced to any major extent for a significant time. The behaviour of our irradiation chimaeras agrees with this observation. Four weeks after grafting, animals possessed T and B cells of donor origin but continued to behave as normal hosts with respect to ease of tolerance induction<sup>9</sup>. We have demonstrated that even 6 weeks is insufficient for functional reconstitution but after 12 weeks (Table 1) the behaviour of the chimaeras was now almost totally like the donor as to tolerance induction.

These results provide strong confirmation of the existence of a decision making event at the level of the macrophage that determines whether in a particular strain of mice the injection of an antigen such as BGG will result in tolerance or immunity.

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#### Separation of T effector cells in humoral and cellular immunity

THE precise relationship between thymus-derived (T) helper cells in the antibody response and T effector cells in cellular immunity is not clear. Both cell functions have closely related dose response curves<sup>1</sup>, kinetics of sensitisation<sup>1</sup>, susceptibility to antibody suppression<sup>1-3</sup> and anatomical distribution<sup>4</sup>. Bone marrow-derived (B) lymphocytes can be stimulated for antibody synthesis by a stimulus from immunocompetent allogeneic<sup>5</sup> or xenogeneic<sup>6</sup> lymphoid cells. These observations have led to the hypothesis that T helper cells are identical to cytotoxic T cells and act on B cells by secreting a factor which stimulates them to produce antibody. Liew and Parish have reported, however, that T helper cells in humoral immunity recognise different forms of chemically modified flagellin than do T cells in cellular immunity. Furthermore, Dennert<sup>8</sup> has shown that T-helper and T-killer cells are mutually exclusive under certain immunisation conditions. The reports of Dennert<sup>8</sup> and Liew and Parish<sup>7</sup> suggest that T helper cells may not be identical to T effector cells in cellular immunity. We have now used a rosette technique9 which distinguishes different degrees of antigen-binding, in combination with velocity sedimentation at unit gravity<sup>10</sup> to distinguish and separate T cells involved in the helper effect from T cells participating in cellular immunity reactions.

We have shown (work in preparation) that sheep red blood cells (SRBC) in complete Freund's adjuvant (CFA) injected C57BL/6 mice (Canadian Breeding Laboratories) induce both humoral and cellular immune reactions within 9 d after immunisation. For both of these responses T cells are necessary: T-helper cells trigger B cells for antibody synthesis<sup>11</sup>; T cells in cellular immunity are involved in delayed hypersensitivity (DHR) and in vitro cytotoxicity (our work in preparation). This system was therefore useful for investigating the relationship between T cells in humoral and cellular immunity in the same experimental animals and within the same lymphoid organ.

DHR and helper cell activity were assayed by adoptive transfer systems as reported previously (our work in preparation). To detect DHR activity, immune cells were injected with 5×107 SRBC intradermally into one footpad of normal animals. The other footpad received SRBC alone. The percentage in footpad volume after challenge was proportional to the number of immune cells injected. The DHR activity of SRBC immune cells was antigen-specific, sensitive to anti-θ serum plus complement, but insensitive to anti- $\theta$  serum alone (our work in preparation).

To assay for helper cell activity, immune cells and 2×108 SRBC were injected with or without  $2 \times 10^7$  bone marrow cells into lethally irradiated recipients and the number of 19S plaqueforming cells (PFC) per spleen was determined 8 d later. An increase in the number of splenic PFC when an immune cell population was injected with bone marrow cells indicated helper cell activity in that population.

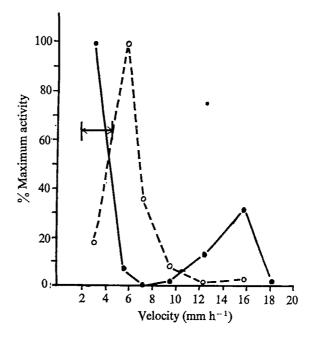


Fig. 1 Sedimentation velocity profiles of 19S helper cells and DHR effector cells from immune lymph nodes. C57BL/6 mice received an intradermal injection of 5×107 SRBC in CFA in the footpad. After 9 d, lymph node cells were removed, and subjected to rosette formation and sedimented with azide present. To assay for DHR, each fraction was injected with SRBC into the footpad of normal animals and the percentage increase in footpad volume was determined after 18 h. Footpad volume was measured by the deflection observed when the mouse foot was immersed in mercury on a Mettler 1600 balance to a mark made immediately distal to the lateral malleolus as described previously (our work in preparation). Three readings were made on each footpad and averaged. The difference in readings before and after skin testing was proportional to the increase in footpad volume. Percentage increase in footpad volume was calculated by dividing the difference by the original reading and multiplying by 100%. The relative activity was determined from a dose-response curve obtained with unfractionated immune cell1 (our work in preparation). To assay for helper cell activity, each fraction with 2 × 108 SRBC was injected either alone or with 2×107 normal bone marrow cells into syngeneic irradiated (750 r.) mice. 198 PFC per spleen were assayed 8 d later. Helper cell activity was calculated by subtracting the number of splenic PFC in mice injected with that fraction alone from that of the fraction plus bone marrow cells. O, 198 helper cell activity; , DHR activity. The velocity range of small lymphocytes ( $\longleftrightarrow$ ) has been indicated for convenience.

19S helper cells sedimented as medium lymphocytes at 5-7 mm h<sup>-1</sup> (Fig. 1). DHR effector cells sedimented over a wider range of velocities (3-7 mm h<sup>-1</sup>), indicating the presence of both small and medium lymphocytes. When immune cells were subjected to rosette formation before sedimenting in the presence of sodium azide (to stabilise T rosettes9,13), rosettes sedimented at velocities greater than 6.5 mm h<sup>-1</sup>, as reported<sup>13</sup>. Rosette formation, however, had no effect on the sedimentation velocity of 19S helper cells (Fig. 1). DHR activity remained in the nonrosette forming small lymphocyte region (3 mm h<sup>-1</sup>) (Fig. 1). Activity in the medium lymphocyte region, however, shifted to the higher velocity region (13-15 mm h<sup>-1</sup>), indicating that medium lymphocyte effector cells had formed rosettes. Without azide in the sedimentation medium T rosettes dissociated and medium lymphocyte effector cells sedimented as single cells (5-7 mm h<sup>-1</sup>). The calculated number of SRBC (bound to make a medium lymphocyte RFC sediment at  $13-15~\text{mm h}^{-1}$ ) is 8-10~SRBC (our work in preparation). Those antigen-binding properties are characteristic of T-type RFC (ref. 9).

These results indicate that 19S helper cells in immune animals are distinct from DHR effector cells. The latter were of two types: (a) non-rosette forming small lymphocytes and (b) medium lymphocyte T-type RFC. We have also found that SRBCspecific effector cells in in vitro cytotoxicity against sheep fibroblasts have the same size, antigen-binding properties, density

and sensitivity to anti-θ serum plus complement as DHR effector cells. These findings indicate that under the conditions of rosette formation used in the present study, 19S helper cells were distinct from T effector cells in cellular immunity.

Since the helper cell assay takes 8 d while the DHR and in vitro cytotoxicity assays take only 24 h, maturation of T helper precursor cells to T helper cells could occur. These experiments, however, strongly suggest that helper cells (or precursors) and T effector cells in cellular immunity are two different cells.

Recently, subsets of T cells with specific functions have been described. Cantor and Asofsky14 have shown that two T cells with different anatomical distribution and sensitive to antilymphocyte serum synergise in the graft-versus-host reaction. Bach et al.15 demonstrated that T cells involved in the mixed lymphocyte reaction are distinct from cytotoxic T cells. The distinction between 19S T-helper cells and T-effector cells in cellular immunity in the present study suggests a further division in T-cell subsets which is characterised in part by different abilities to bind antigen.

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#### Cell-mediated immunity to Epstein-Barr virus and a blocking factor in patients with infectious mononucleosis

ALTHOUGH the humoral immune response to Epstein-Barr virus (EBV) in infectious mononucleosis (IM) has been studied extensively1,2, little is known about cell-mediated immunity (CMI) to EBV in the disease. We describe here the status of CMI to EBV, and the presence of a blocking factor which can abrogate leukocyte migration inhibition, in patients with IM.

Serum antibody to EBV-capsid antigen in normal adults and patients with IM were measured by indirect immunofluorescence<sup>3</sup>, using SH-RP cells<sup>4</sup> previously irradiated with ultraviolet light to enhance virus capsid antigen5. The results are shown in Tables 1 and 2.

Leukocyte migration inhibition was performed by employing 20 µl capillary tubes (Drummond Scientific Co., USA) as described earlier<sup>6</sup>. Peripheral leukocytes from each individual were washed four times in Hanks' balanced salt solution and then adjusted to  $3\times10^{-7}$  cells ml<sup>-1</sup> in Eagle's basal medium (Grand Island Biological, USA) with 10% heat-inactivated foetal bovine serum (BioCult, UK). The capillaries were filled with the cell suspension, sealed at one end and centrifuged at 950g for 5 min. These were cut at the cell-medium interface and immersed in the medium to which had been added an EBV-antigen preparation from P3HR-1 cell lysate<sup>6</sup>. The final concentration of the EBV-antigen preparation in the medium was 1:5. A minimum of eight replicate cultures was set up for each sample. Similarly, control cultures were set up in medium without EBV-antigen preparation. The cultures were incubated at 37° C in a humidified atmosphere of 5% carbon dioxide for 16 h.

The extent of leukocyte migration was determined by multiplying the greatest diameter of the area of cell migration by its perpendicular diameter measured by an ocular micrometer and the percentage inhibition was calculated as:

$$1 - \frac{\text{(mean extent of migration with antigen}}{\text{mean extent of migration without antigen}} \times 100$$

From the distribution of results (Table 1), we have considered a response positive when the percentage inhibition is 20% or greater, negative if smaller than 15% and equivocal if in between.

To test whether the effects of the EBV-antigen preparation on leukocyte migration could be abrogated by serum, sera from normal individuals (which had been stored at  $-20^{\circ}$  C for up to 8 weeks) and from subjects (cases 1, 2 and 3) at the acute and the convalescent phase of IM (fresh sera) were used. One ml of serum at various concentrations was mixed with an equal amount of the EBV-antigen preparation. The mixture was incubated at 37° C for 30 min. Leukocyte migration inhibition was carried out as described, using as test cells peripheral leukocytes (from a normal adult, case 7) known to have their migration inhibited by the EBV-antigen preparation in the presence of foetal bovine serum alone. The final concentration of EBV preparation in the serum-antigen mixture used in the medium was 1:5. EBV-antigen preparation and serum put up separately at the same concentration in the medium were used as controls. The results of these experiments are listed in Table 2.

In addition, the acute sera (stored at  $-20^{\circ}$  C for 14 d) and the freshly collected convalescent sera from cases 1, 2 and 3 were used to abrogate the effect of EBV-antigen preparation on leukocyte migration using autologous and heterologous leukocytes collected at the convalescent phase of IM. The results of these experiments showed that acute and convalescent sera from all three cases blocked the EBV-induced migration inhibition of autologous and heterologous leukocytes. In addition, the blocking effect observed decreased with increasing dilution of serum. Leukocytes and sera from cases 4 and 5 (Table 1) were used in a similar experiment, in which the antigen specificity of the blocking effect was indicated, using Escherichia coli as the irrelevant antigen (Table 3).

Acute sera from cases 1 and 4 were fractionated in a Sephadex G-200 column (2.5×100 cm, Pharmacia, Uppsala) at a flow rate of 20 mlh<sup>-1</sup>. The IgG and IgM fractions were collected and examined at 28 nm in a Beckman spectrophotometer.

Immunodiffusion of these fractions against standard rabbit anti-human IgG and anti-human IgM (Behringwerke,) in 0.8% agarose (Seakem Agarose, Marine Colloids Inc., USA) buffered at pH 7.6 showed that the IgG fraction reacted with the antihuman IgG but not with anti-human IgM standard serum. Similarly, the IgM fraction reacted with anti-human IgM standard serum only. Immunofluorescent testing showed that both fractions contained antibodies to the capsid antigen of EBV. Blocking activity to the EBV antigen preparation was carried out as described and was detected only in the IgG fraction.

Table 1 Serum antibody to EBV-capsid antigen and CMI to EBV detectable by leukocyte migration inhibition

Ca		Serum- antibody titres to EBV-capsid	Paul- Bunnell titre	de leuko	I to EBV as etected by cyte migration thibition†
1	IM acute	antigen* 320	896		$(-2.3\pm3.1)$
	convalescent‡	320	_	+	$(72.1 \pm 2.5)$
2	IM acute	80	55	+	$(15.8 \pm 3.8)$
	convalescent‡	160	+	+	$(27.0\pm1.3)$
3	IM acute	80	112		$(-11.9\pm1.7)$
	convalescent!	40		****	$(26.0\pm1.2)$
4	IM acute§	160	+ 11		$(2.0\pm3.1)$
5	IM convalescent§	40	-11	+	$(20.0 \pm 2.5)$
6	Normal	20	••	+	$(31.0\pm4.3)$
7	Normal	160		÷	(24.0 + 2.1)
8	Normal	40		<u> </u>	$(35.0 \pm 2.6)$
9	Normal	80		÷	(42.5 + 2.5)
10	Normal	320		÷	$(34.0 \pm 1.3)$
11	Normal neonate	0			$(5.0\pm3.0)$

\*Assayed by immunofluorescence3 using SH-RP cells4.5

†Mean % inhibition  $\pm$  s.e. as determined from a minimum of eight replicate cultures. 20% or greater is marked as +, less than 15\% as -, and in between as  $\pm$ .

‡Collected 10-14 d after collection of acute sera, at convalescent phase of the illness; no Paul-Bunnell heterophil antibodies present. §Tests performed in 10% equine rather than foetal bovine serum. ||Mono-spot test.

CMI is important in defence against infection with most viruses, including oncogenic viruses8,9. As well as causing IM, EBV is a potential oncogenic virus in man because of its ability to transform human haematopoietic cells in vitro4.10, its ability to cause lymphomas in non-human primates11,12 and its association with at least two types of human tumour, Burkitt's lymphoma<sup>13</sup>, and nasopharyngeal carcinoma<sup>14</sup>.

We have shown that CMI to EBV was not detected in vitro in circulating leukocytes in the acute phase of IM (Table 1), but was found in circulating leukocytes of normal adults who had antibody to EBV and patients convalescent from IM. In

Table 2 Blocking effect by sera from patients with IM on leukocyte migration inhibition and its absence in normal sera

	Serum anti-		% Inh	nibition*
Case	EBV capsid	S	erum alone	Serum + EBV
no.	antibody			
	titre			
1 acute†	320	_	$(-3.2\pm2.7)$	$-\parallel (-4.8\pm 2.4)$
convalescent‡	320	_	$(5.2\pm1.3)$	$-\parallel$ ( 8.3±2.1)
2 acute†	80	_	$(-3.3\pm0.8)$	$-\parallel$ ( 11.0±2.1)
convalescent‡	160	_	$(1.0\pm1.8)$	$-\parallel (-12.0\pm 2.3)$
3 acute†	80	-	$(12.3\pm0.5)$	$-\parallel (6.2\pm 2.4)$
convalescent‡	40	-	$(4.1\pm0.8)$	$-\parallel (10.7\pm2.9)$
6§	20	-	$(-1.4\pm 2.5)$	$+ (31.0 \pm 4.3)$
7§ 8§ 9§	160	· <b>-</b> -	$(-8.7\pm1.4)$	$+ (24.0\pm2.1)$
8§	40	_	$(8.0\pm1.3)$	$+ (33.0 \pm 1.8)$
9§	80	_	$(11.3\pm1.9)$	$+$ (39.4 $\pm$ 1.2)
10§	320	_	$(-3.4\pm2.0)$	+ ( 33.6±0.7)
21§	160	_	$(12.2\pm1.5)$	$+ (26.3\pm2.4)$
28§	80	_	$(12.3\pm3.1)$	$+ (32.7\pm2.8)$
40§	40	-	$(5.3\pm2.4)$	$+$ (31.3 $\pm$ 2.7)
51 §	40	_	$(13.2\pm1.4)$	$+$ (31.4 $\pm$ 1.6)
72§	20	_	$(-2.4\pm1.1)$	$+$ ( 23.1 $\pm$ 2.3)

\*Leukocyte migration inhibition was determined using leukocytes The usocyte migration inhibition was determined using leukocytes (from a normal subject, case 7) whose migration is known to be inhibited by the EBV-antigen preparation in the presence of foetal bovine serum alone. % inhibition was  $24.0\pm2.1$ . Mean of % inhibition  $\pm$  s.e. was derived from eight replicate cultures. Less than 15% inhibition is marked as -, 20% or greater as +. †Freshly-collected acute IM serum with EBV capsid antibody and Paul-Bunnell heterophil antibodies as in Table 1.

‡Freshly-collected convalescent IM serum with EBV capsid antibody but no Paul-Bunnell heterophil antibody.

§Serum collected from normal adults and stored at -20° C for 6-8 weeks before use

||Indicates abrogation of expected leukocyte migration inhibition.

addition, a blocking factor specific to EBV was found in the acute and convalescent sera of these patients (Tables 2 and 3).

Haider et al.15 have reported that in vivo CMI to tuberculin was apparently depressed in patients with acute IM. The absence of CMI to EBV in circulating leukocytes in the acute phase of the disease in the present study still allows the possibility that leukocytes mediating CMI to EBV are active outside the circulation. The development of CMI to EBV at the convalescent phase, however, as detected by leukocyte migration inhibition in the absence of autologous serum, suggests that a transient impairment of CMI function might have occurred.

Table 3 Effect of sera from patients with IM on leukocyte migration inhibition induced by EBV or E. coli

		-						
	Serum % Inhibition*							
	anti-	Leukocyt		Leukocyt	es from			
	EBV	acute IM	(case 4)	convales	cent IM			
Serum	capsid		•	(case 5)				
	anti-	EBV	E. coli†	EBV	E. coli†			
	body	antigen	antigen	antigen	antigen			
	titre	_	_	-	•			
Equine alone	0	$2 \pm 3.1$	$28 \pm 0.5$	20:: 2.5	38 - 3.1			
Normal human								
adult	80	$-3 \pm 3.4$	$38 \pm 1.0$	$23 \pm 2.2$	$41 \pm 2.7$			
Case 4	160	$-2 \pm 3.4$	$31 \pm 4.0$	$5 \pm 1.0$	$36 \pm 3.3$			
Case 5	40	$-5 \pm 4.0$	$29 \pm 3.1$	2 4.5	$44 \pm 3.0$			

% Inhibition is expressed as mean  $\pm$  s.e. derived from a minimum of four replicate cultures.

†5.0×107 organisms ml<sup>-1</sup> prepared according to the method of Fimmel and Keast7.

It is not certain whether the blocking factor detected is part of normal negative feedback mechanisms involved in the immunity to virus infections, or whether it has an important role in the differential pathogenesis of diseases caused by EBV. The nature and the in vivo halflife of this blocking factor are unknown although we have shown that it was in the IgG fraction. Our experiments also indicate that it is not related to the EBV-capsid antibody, nor the Paul-Bunnell heterophil antibodies, as it is absent in normal sera with high level of EBVcapsid antibody (cases 7 and 10) and present in convalescent sera with no Paul-Bunnell heterophil antibodies. This could mean that the capsid antigen of EBV is not the antigen involved in the initiation of leukocyte migration inhibition, but as yet we have no direct evidence on this point.

Dameshek<sup>16</sup> has proposed that infectious mononucleosis, a benign lymphoproliferative illness, is a form of 'self-limiting' malignancy. It is plausible that CMI to EBV detected in these individuals is responsible for this 'self-limiting' expression. Considering the strong association of EBV with Burkitt's lymphoma and nasopharyngeal carcinoma it is important that CMI to EBV and the blocking factor be investigated in patients with these diseases at remission and recurrence.

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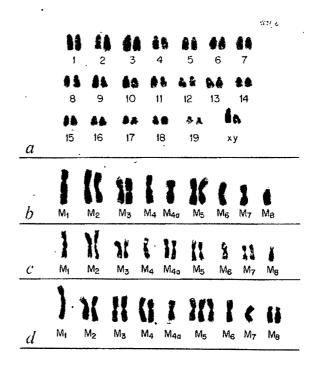
#### Reversible appearance of a specific chromosome which suppresses malignancy

MALIGNANT cell transformation is associated with the appearance of abnormal chromosome patterns1. The degree to which malignant potential is expressed by transformed cells seems to depend on the balance between specific chromosomes responsible for the expression or suppression of malignancy<sup>2</sup>. Sachs et al.2-4 have demonstrated a relationship between these chromosomes and both oncogenic activity and cell membrane characteristics in transformed and reverted cells. Benedict et al.5, identified specific chromosomes associated with either expression or suppression of malignancy in hamster fibrosarcoma cells; tumorigenicity in different clones was related to the balance of these chromosomes.

We examined the genetic constitution of an established tumour line, and the chromosome alterations which may develop, by karyotypic analysis of 122 metaphases in cells from cultures and tumour nodules. Table 1 shows that prolonged maintenance of MC-42 fibrosarcoma cells in tissue culture has no significant effect on the modal number of chromosomes, when compared with the number in cells from serially-transplanted tumours. Injecting these cultured cells into syngeneic hosts resulted in a slight decrease in the modal number of chromosomes, irrespective of the duration of in vivo propagation. In all cases, chromosome banding revealed a complete complement of normal mouse chromosomes (Fig. 1a) in both cultured and serially-transplanted tumour cells. Eleven to sixteen subtelocentric, submetacentric and metacentric marker chromosomes, were also present; the cells inconsistently gained normal mouse chromosomes, as well as a long telocentric chromosome (Figs 1 and 2).

The only consistent genetic alteration associated with the culturing of MC-42 cells appeared in the marker chromosome pattern which is characteristic of this tumour line (Figs 1 and 2). The eighth marker chromosome (M<sub>8</sub>) showed specific variations between cells from tumour nodules and those from cultures. All cells from subcutaneous tumours contained only one M8 chromosome. When the tumour was transferred to tissue culture, however, cells with two M<sub>8</sub> chromosomes evolved,

first appearing (1 of 16 cells) in the 6th week of tissue culture propagation (Table 1). Four weeks later the proportion increased to 36% (4 of 11), and to 50% (11 of 22) after 12 and 17 weeks. Thereafter, the ratio of cells with one M<sub>8</sub> to those with two remained 1:1, regardless of the duration for which the cells had been cultured (not shown in Table 1). None of the seven other groups of marker chromosomes observed in MC-42 tumour cells showed any consistent variation associated with in vitro propagation (Figs 1 and 2).



Chromosome banding patterns: a, Normal C3H/HeJ mouse cells. b, Marker chromosomes from a serially-transplanted tumour. Note the eighth marker (M<sub>8</sub>); all cells from growing MC-42 tumours contain only one M<sub>8</sub> chromosome. c, Marker chromosomes from a cultured tumour cell containing one M<sub>8</sub> chromosome. d, Markers from a cultured tumour cell containing two M<sub>8</sub>. The tumour studied, a methylcholanthrene-induced fibrosarcoma (MC-42), had been propagated by serial transplantation in C3H/HeJ mice and had never been maintained in culture. The growth and immunogenicity of this tumour line have been described previously<sup>6,7</sup>. All tumours in this study were produced by subcutaneous inoculation of 20,000 viable cells (trypan blue exclusion) in McCoy's medium. Cultured cells were maintained in McCoy's 5a medium with 13% foetal calf serum, supplemented with penicillin, streptomycin and HEPES buffer. Cells were incubated in colchicine for 4 h, treated with trypsin and incubated in 1% sodium citrate for 20 min. They were then exposed to a fixative solution (acetic acid:methanol, 1:3) resuspended in 75% acetic acid and prepared for staining with 2% aceto-orcein. The method of trypsin/Giemsa R66 banding8 of chromosomes was used.

Tissue culture clones composed entirely of cells having either one or two M<sub>8</sub> marker chromosomes were grown by seeding cells from mixed cultures on to glass chips in Leighton tubes. Mass cultures containing equal proportions of cells with one and two  $M_8$  consistently produced a significantly greater number of clones of the one M8 variety. Multiple samples from clones, taken at periodic intervals, showed complete homogeneity with respect to either the single or duplicate M<sub>8</sub> pattern; no clones older than 6 weeks were used. When cloned cells having two M<sub>8</sub> were reimplanted into syngeneic hosts, the resulting tumour nodules were composed only of cells with one M<sub>8</sub> chromosome (Fig. 2). Inoculations of cloned cells having one M<sub>8</sub>, as well as inoculations of mass cultures, similarly produced tumours containing only cells with one M8 chromosome in every case. Cells with the duplicate M<sub>8</sub> pattern did not appear in vivo, irrespective of the age of the cultures

inoculated, the size of the tumour nodule examined, or the duration of in vivo growth. When cells from various (mass) cultures were reimplanted into syngeneic hosts, the frequency of tumour formation was lower if the inoculum contained a greater proportion of cells with two  $M_8$  chromosomes (see Table 1). No other consistent alterations in chromosomes were observed when cloned cells were injected into mice, although an occasional gain of markers  $M_{4a}$  or  $M_6$ , or loss of an  $M_2$  or  $M_5$ , was inconsistently noted.

Therefore, although cells with two  $M_8$  seem as viable as those with one  $M_8$  in vitro, the capacity for tumour growth is expressed only by those which have lost the second  $M_8$ .

Lymphocytes from mice bearing serially-transplanted tumours (all containing one  $M_8$ ), compared with those from mice bearing tumours (of equal size) which had been produced by inoculating (9 week) mass cultured cells, are more cytotoxic to tumour cells in each of the four target cell groups (Fig. 3). This difference was not affected by the addition of serum from mice with the tumours. On the other hand, target cells were more efficiently lysed if they had been maintained (9 weeks) in tissue culture. Cells from clones with one  $M_8$  were destroyed as efficiently as those from mass cultures. In contrast, the cytotoxic effect of sensitised lymphocytes was significantly less when target cells with two  $M_8$  chromosomes were used.

1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	111 P1 1 Q1 Q X 1 M <sub>1</sub> M <sub>2</sub> M <sub>3</sub> M <sub>4</sub> M <sub>5</sub> M <sub>6</sub> M <sub>7</sub> M <sub>8</sub>							
M <sub>1</sub> M <sub>2</sub> M <sub>3</sub> M <sub>4</sub> M <sub>4o</sub> M <sub>5</sub> M <sub>6</sub> M <sub>8</sub> C	M, M <sub>2</sub> M <sub>3</sub> M <sub>4</sub> M <sub>40</sub> M <sub>5</sub> M <sub>6</sub> M <sub>8</sub>							
M <sub>1</sub> M <sub>2</sub> M <sub>3</sub> M <sub>4</sub> M <sub>4</sub> M <sub>5</sub> M <sub>6</sub> M <sub>8</sub> e	M <sub>1</sub> M <sub>2</sub> M <sub>3</sub> M <sub>4</sub> M <sub>40</sub> M <sub>5</sub> M <sub>6</sub> M <sub>8</sub> f							

Fig. 2 Marker chromosome patterns: a, Tumour cell from a 10 week old mass culture. b, Cell from a tumour produced by injecting 20,000 (mass) cultured cells into a C3H/HeJ mouse. All cells in such tumours contain only one M<sub>8</sub> chromosome. c, Tissue culture clone (c1-7) containing only cells with two M<sub>8</sub>. d, Tumour nodule grown by injecting cells from the cultured clone c1-7 (shown in c). e, Tissue culture clone (c1-2) containing only cells with one M<sub>8</sub>. f, Tumour produced by subcutaneous inoculation of cells from clone c1-2 (shown in e).

The decreased ability of mass cultures of tumour cells containing both cells with one and two  $M_8$  to sensitise lymphocytes, together with the significantly lower cytotoxicity of target cells containing two  $M_8$ , may reflect the failure of cells with two  $M_8$  to express their tumour-associated antigens. The lower cytotoxicity does not seem to be directly related to the culturing process, as cells from mass cultures and from clones with one  $M_8$  were more efficiently lysed than cells from serially-transplanted tumours.

Giemsa banding of chromosomes (Fig. 1) shows that the M<sub>8</sub> has no bands on the short arms, suggesting that this portion of the chromosome may be from the normal mouse chromosome 19. The centromere is heavily stained and the long arms contain three prominent, moderately-stained bands in the mid-region, indicating that this portion may be an unaltered number 7 chromosome. Karyotypic studies of cells containing two M<sub>8</sub> consistently suggest that the M<sub>8</sub> is derived from a deleted number 19 chromosome, translocated on to a number 7. All MC-42 cells contain a complete complement of normal chromosomes together with an inconsistent number of additional chromosomes.

Multinucleated cells were present in all cultures before the duplicate M<sub>8</sub> pattern occurred. None of the cells displayed asynchronous DNA synthesis (which might indicate cell fusion) after exposure to <sup>3</sup>H-thymidine for 5 h. This suggests that multinucleation, chromosome ploidy and the evolution of MC-42 cells with two M<sub>8</sub> chromosomes may be the result of mitotic abnormalities, rather than of cell fusion.

Our data, therefore, show a strong association between the appearance of a second M<sub>8</sub> marker chromosome in MC-42 fibrosarcoma cells and the suppression of malignant characteristics. Sachs et al.1 have found that the in vitro propagation of polyoma virus-transformed cells may favour the appearance of chromosomes which suppress malignant characteristics. By fusing highly malignant ascites tumour cells with non-malignant cells, Harris et al.10 produced both tumour cells in which malignancy and the expression of histocompatibility antigens were suppressed; a loss of chromosomes from these hybrid cells was associated with recovery of the ability to grow progressively in vivo. These reports are consistent with our data, in that cultures of the MC-42 fibrosarcoma developed variants which appeared less tumorigenic and less immunogenic, and which lost a chromosome when malignancy was expressed. The MC-42 tumour line, however, contains the only example of a suppressor chromosome (the second M<sub>8</sub>) which is paired with a morphologically identical chromosome (M<sub>8</sub>) that alone has no apparent direct influence on the suppression of malignancy. In polyoma virus-transformed hamster cells11, where the chromosome group which suppresses malignancy has been identified, there seems to be a direct relationship between the number of suppressor

1	.,		1 Chro									<del></del> ,			
Cell	Tumour diameter	Age of culture			Cells v	vith ch	romoso	me nu	mber =	<b>=</b>		No o	f cells	No. of r	
source	(mm)	(weeks)	48–51	52-53	54-55	56–57	58–59	60-61	62-63	64–72	106	contai		no. of n	nice
Serial transplant	12 34	_	1			3	5	2 3	1			11 4	0		
Tumour culture	_ _ _	6 10 12 17		1 4 2	1	3 5 4 9	6 4 5 1	3	1	2	1	15 11 5 6	1 4 5 6	N.D. 6/6 3/6 3/6	
Cultured cells (in vivo)	4 17 20 25 26 28 29 31	   	1 1 2	2 2 2 1 1	1 4 2 2 2 6 2 6	2 5 1 1 2 2	3	1				3 16 4 2 5 12 3 9	0 0 0 0 0 0		,

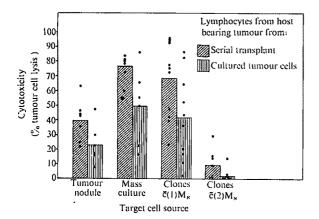


Fig. 3 Ability of lymphocytes from mice administered seriallytransplanted or (mass) cultured tumour cells to lyse target cells from either serially-transplanted tumours, mass cultures, clones of cells with one M<sub>8</sub> or those with two M<sub>8</sub>. Lymphocytes were purified (Ficoll-hypaque separation) from the spleens of animals bearing palpable tumours and the cytotoxicity assay was performed according to the method described previously Target cells were plated in Falcon 3040 Microtest II wells and the cells directly counted 24 h later. Target cells were then exposed to lymphocytes for 2 d, washed with saline and stained with crystal violet for final counting.

chromosomes and the degree of suppression. Furthermore, a chromosome group responsible for the expression of malignancy has been identified in that tumour, the number of such chromosomes in cells of any clone being proportional to the degree of malignancy of that clone. When cells from clones of suppressed polyoma virus-transformed cells are reimplanted into syngeneic hosts, the tumours produced contain fewer suppressor chromosomes and more chromosomes for the expression of malignancy. Careful examination of MC-42 tumour cells failed to demonstrate a specific chromosome group responsible for the expression of malignancy.

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#### Isolation of Bacillus anthracis from an aborted bovine foetus

We report here the presence of virulent anthrax bacilli in aborted tissues. This is significant in that it suggests that the foetus becomes infected during the bacteraemia/pyrexia phase of the dam, and that the bacilli continue to multiply in and cause the death of the foetus despite the recovery with treatment in the parent cow. Since abortion may be a sequel to the infection in pregnant animals which survive, the products of such abortions must be regarded as potential vectors of anthrax both for man and other animals.

In Spring 1974 two cows died within 24 h of each other and anthrax was confirmed in each case. Control measures included the immediate vaccination of the remainder of the herd with a proprietary spore vaccine. Anthrax Spore Vaccine (Living), (Wellcome). The following day a cow became ill, having a rectal temperature which rose to 41.1° C. Antibiotic therapy was instituted and the animal recovered within 48 h. But 13 d after vaccination this cow aborted a foetus and placenta within an isolation box. In keeping with the Brucellosis Incentives Scheme rules, serum, milk and the products of abortion were forwarded to the local Veterinary Investigation Centre. The gestational age of the foetus was determined as 120 d. but apart from softening of the liver no gross pathological changes were seen in either the foetus or the placenta which might have indicated the possible presence of anthrax. Examination of smears prepared from foetal stomach contents and placental cotyledons revealed chains of encapsulated bacilli; overnight cultures prepared from similar sites were suggestive of Bacillus anthracis and material was forwarded to the Central Veterinary Laboratory, Weybridge, for identification.

Two guinea pigs inoculated subcutaneously with 1 ml of a saline suspension of the culture ( $\sim 1 \times 10^6$  organisms) and 1/100 dilution of a saline suspension ( $\sim 1 \times 10^4$  organisms) respectively died within 48 h. On post mortem examination hyperaemia associated with oedema, characteristic of anthrax, was observed and Bacillus anthracis was isolated in pure culture from blood and oedema. Anthrax was confirmed by the presence of encapsulated bacilli in guinea pig blood smears fixed and stained with 1% aqueous solution of polychrome methylene blue. The vaccine strain is seldom lethal for guineapigs and is not encapsulated if present in the blood of inoculated guinea pigs1.

Two vaginal swabs and a faeces sample, taken from the cow 3 d after abortion, did not yield anthrax bacilli on bacteriological examination at the Central Veterinary Laboratory.

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#### Erratum

In the article "Precocious development of detoxicating enzymes following pituitary graft" by G. J. Wishart and G. J. Dutton (Nature, 252, 408; 1974) line 3 in paragraph 10 should read '... similar weight from 2-d chicks down to 18-d embryos,' and not as printed.

# matters arising

#### **Evolution of the Tasman Sea**

WE do not accept Hayes and Ringis" "conclusive evidence" that the central Tasman Sea evolved by symmetrical spreading from a median north-west trending ridge between 80-60 Myr ago, for several reasons.

First, their 'ridge' and 'fracture zones', which are mantled by sediments, have no bathymetric expression.

Second, they ignore the only conspicuous topographic features within the limits of their magnetic anomaly

pattern. These are the seamounts and knolls respectively north and southwest of Gascovne Guyot. Both trends are oblique to their magnetic grain and fracture direction (Fig. 1).

Third, their seismic data are said to "reveal a distinct basement ridge" blanketed by acoustically transparent sediments which "thin subtly near the crest of the buried ridge". We contend that only their profile X could be described thus, and its "axial rift" is equivocal.

Fourth, they ignore an elongate area of thin sediments' which is coaxial

with the knolls south-west of Gascoyne Guyot (Fig. 1), but which is unrelated spatially to the 'ridge' and 'fracture zones'.

Fifth, they are prepared to consider that spreading "was much slower in the northern area than in the southern area", but this is contrary to the anomaly spacings, and therefore to the identifications, which they adopt.

Sixth, they maintain that limited subduction along the Tasman margin of Australia 80-60 Myr ago cannot be excluded. Yet no rocks of orogenic character on this margin are younger than Triassic and all are related to a Palaeozoic-Mesozoic tectonic regime expressed in a north-north-west continental grain which is cut obliquely by the present margin. The only post-Triassic volcanics on this margin are non-orogenic and are less than 55 Myr old3.

Seventh, their pre-Tasman reconstruction of Australia/Antartica and the New Zealand Plateau involves a misfit (gaps plus overlaps) which is equivalent to half the area of the latter. Furthermore, they reduce the potential misfit considerably by eliminating the Alpine Fault, although most studies indicate that major movement on it occurred during the early Cretaceous'.

Eighth, their hypothesis cannot account for the NE-SW orientation (oblique to the trends of their 'ridge' and 'fracture zone'; Fig. 1) of the dominant topographic elements of the Tasman margin, centred on Sydney.

Finally, their hypothesis cannot account for recurrent Cainozoic basaltic volcanism on Australia's Tasman margin3 which entirely post-dates, their postulated termination of Tasman dilation 60 Myr ago.

A more extensive discussion of these points can be obtained from us.

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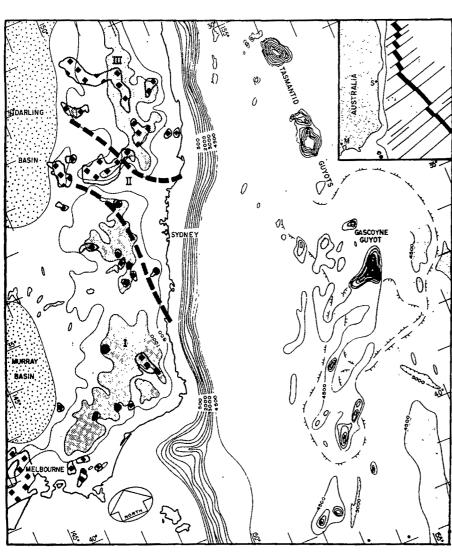


Fig. 1 Tasman margin of mainland Australia. Contour interval at 500 m. Diamond stipple, Cainozoic volcanics; roman numerals, geological provinces of the pre-Tasman tectonic regime: I, early Palaeozoic; II, late Palaeozoic-early Mesozoic; III, mid-Palaeozoic to early Mesozoic. Broken lines, approximate 600 m sediment isopach, with barbs indicating direction of thickening. Bathymetry after Hayes and Connolly and Connolly Sediment isopach from Houtz et al. Insert shows spreading ridge and fracture zone trends of Hayes and Ringis1.

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#### Red Sea spreading

GIRDLER and Styles1 cite geological evidence2 from the Gulf of Suez area favouring the idea that a Carboniferous Red Sea depression existed. Various views have been put forward concerning the age of the Suez rift structures3-6. Some authorities believe block faulting and rifting does not predate Oligocene6; others have isopached a post-crystalline Basement Complex to pre-Permian linear trough7; and some believe in a Mesozoic trough3.

Even if definite proof were available publicly of a Carboniferous trough this would not necessarily prove that a trough of this age existed in the Red Sea area, because the Suez structures are part of a failed arm system8,9; the Red Sea structures are part of an active spreading ridge system and the Aqaba-Dead Sea structures part of an active transform system. As such the three arms have had interlinked but separate geological histories8.

There is clear evidence in the southern Red Sea area, however, of major rifting before the Early and Medial Eocene proposed by Girdler and Styles<sup>1</sup>. A pre-Mesozoic (?pre-mid Jurassic) trough some 500 km long; up to 150 km wide and containing up to 7.5 km of sediments in the Mandera region of Somalia extends from coastal Tanzania to Afar<sup>10</sup>.

This trough as roughly mapped intersects the Cainozoic RRR Afar junction. Some implications are: first, that the Somalia trough may be part of an older failed rrr 'Karroo' junction, with the other failed arms buried beneath the sediments of the southern Red Sea and Gulf of Aden; second, the evolution of the Cainozoic Nubian - Somalian -Arabian plate relationships must be viewed with this in mind; and third, that the Mesozoic African plate may have rotated anticlockwise after the attempted 'Karroo' disruption (crustal thinning, necking, troughing and so on) so enabling the Cainozoic East African Rift to develop over the 'Karroo' mantle plume system.

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DR GIRDLER AND MR STYLES REPLY-WHITEMAN'S views on the formation of the Red Sea are well known. He considers1 that "downwarping has been the major force in shaping the Red Sea depression" and he objects to any major crustal separation with the evolution of oceanic crust.

His objection to a Carboniferous Red Sea Depression is puzzling since in the literature he has been an advocate of this. In the summary of his paper Formation of Red Sea Depression he states "A depression existed in the northern part of the region in Carboniferous times . . . " and on p. 237 we find "In the writer's (Whiteman's) view the Red Sea has been an area of subsidence for a large part of post-Cambrian time." We respect that this is a view held by some geologists and mentioned it in passing2. We were careful to put a question mark<sup>2</sup> in our Fig. 7 and it should be clear from our Figs 5 and 6 that it is unimportant to our arguments.

We have been interested in the postulated 'ancestral pre-Jurassic trough' running from the Southern Red Sea across the horn of Africa to intersect the Tanzania coast<sup>3,4</sup> but at the moment it seems unrelated in time and space to the seafloor spreading discussed.

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#### Solubility of oxygen-nitrogen mixture in water

Maharajh and Walkley1 have reported that mixtures of gases containing oxygen do not behave independently when they are dissolved in water, and as a consequence Henry's law may be

deviated from by as much as 30%. Liss and Slater<sup>2</sup> found that the amount of oxygen dissolved in water from an air mixture at 25° C and atmospheric pressure obeyed Henry's law within their experimental limits (4%). Wilson<sup>3</sup> has reviewed some of the various measurements of oxygen solubility in water at different partial pressures and showed that Henry's law is generally accurate in predicting the amount of gas dissolved to within a few per cent. A thermodynamic analysis4 of Maharajh and Walkley's results showed that the large negative deviation from Henry's law (maximum at about a 1:1 nitrogen to oxygen ratio) leads to an absurd value for the Henry's law constant.

There have been many reports of the solubility of gas mixtures in water, and in most cases these may be shown to be in agreement with Henry's law. Glasstone5, using Winkler's data6, has demonstrated that the solubilities of oxygen, nitrogen and argon each multiplied by their atmospheric partial pressure may be used to calculate the solubility of air in water to within 1%. We have calculated the hypothetical solubility of an air mixture<sup>7</sup> of oxygen, nitrogen, argon and carbon dioxide, using smoothed literature values for the solubilities of the individual gases8,8. The resulting solubility is 4% higher than that reported by Winkler<sup>6</sup>. Behnke<sup>10</sup> measured the solubility of a mixture of argon and nitrogen in water, and his value is 5% above the value calculated from Henry's law and using the recommended literature values for the solubilities of argon and nitrogen8.

We have measured the solubilities in water of nitrogen, oxygen, and a mixture of oxygen (49.5 mol %) and nitrogen (50.5 mol %) at atmospheric pressure and 25° C. The procedure used for the solubility measurements has been desscribed previously11, and the precision of these measurements is  $\pm 1\%$ . The experimentally determined solubilities expressed as mole fractions (at one atmosphere partial pressure of gas) are given in Table 1.

The value predicted for the mixed gases on basis of Henry's law is 0.1711 × 10<sup>-4</sup>, which is 3% lower than the experimental value (average,  $0.1770 \times 10^{-4}$ ). These results are conclusive evidence that Henry's law is not deviated from

Table 1 Solubilities as mole fractions  $\times$  104. Literature<sup>8</sup> values are given in parentheses

Little	o mado uno gini	m paroneneses
Gas	Experiment	Calculated
N <sub>2</sub>	0.1166 0.1166 (0.119)	_
O <sub>2</sub>	0.2261 0.2251 0.2284 (0.231)	_
O <sub>2</sub> /N <sub>2</sub>	0.1775 0.1779 0.1757	0.1711

Girdler, R. W., and Styles, P., Nature, 247, 7 (1974).

<sup>&</sup>lt;sup>2</sup> Heybroek, F. in Salt Basins around Africa, 17 (Institute of Petroleum, 1965)

by as much as Maharajh and Walkley claim. It appears that if anything, the solubility of oxygen in water is enhanced to a small extent by the presence of nitrogen rather than lowered by it.

We have had considerable experience in determining gas solubilities by gas chromatography (the technique Maharajh and Walkley used). It is extremely difficult to attain precisions better than 3% by this method. Indeed, in our judgment, the precision of other workers may actually be as poor as  $\pm 10\%$ . We suggest that the results reported by Maharajh and Walkley are due to inherent difficulties in the gas chromatographic approach.

This work was supported by the National Institute of General Medical Sciences.

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#### Redshift during Pioneer-6 solar occultation unexplained or predicted

THE recent detailed analysis1,2 of Pioneer-6 data<sup>3</sup> demonstrated, under controlled conditions, the existence of the redshift effect which we predicted<sup>4-6</sup>. The explanations proposed by us for certain anomalous redshifts were based on the simple fact, that a moving inhomogeneous medium results in frequency shift and line broadening4.5. (But there is no close correlation between line broadening and frequency shift.) Therefore the search for highly specific or hypothetical explanations for redshift effects, observed on or around the Sun, seems to be unjustified.

The Pioneer-6 frequency residuals could be reproduced well (Fig. 1), without any effort to reach a better agreement between

measured points and theoretical curve. In computing the theoretical curve, the method described in refs 4-6 was used. In the given range of heliocentric distances the particle density was taken proportional to  $r^{-3}$  and the velocity of the medium was decreased in the vicinity of the Sun. Unfortunately the original data of Pioneer-6 were not available to us, so we were compelled to use the data

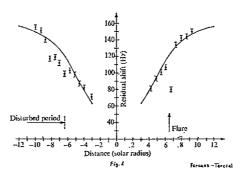


Fig. 1 Residual frequency shift1 and computed curve, corresponding to the moving medium around the Sun, as a function of the minimum distance of Pioneer-6 2,292 MHz radio signal to the centre of the Sun.

published in refs 1-3. From these, however, one cannot establish exactly the zero-line of frequency residuals. Therefore the measured points and the theoretical curve were tied together on the basis of the measured data, referring to  $r = 5 R_{\odot}$ , known satisfactorily from the Taurus A experiments<sup>4</sup>.

Furthermore, the overall picture of solar activity exhibits a correlation with deviations from the theoretical curve, and the effect of enhanced solar activity appears also in the asymmetric bandwidth pattern (ref. 2, Fig. 1b).

With special respect to the opportunities afforded by the space shuttle, it would be expedient in the future to measure accurately the solar disk and corona (the latter by occultational methods) for frequency shift and line broadening in different regions of the electromagnetic spectrum.

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#### **Spatial distribution** of cometary outbursts

PATASHNICK et al.1, in reproducing figures of the position of comets at the times of cometary outbursts from the papers of Pittich2,3, have omitted the outbursts of comet P/Schwassmann-Wachmann (1) (1925 II) which have been observed regularly since 1927 (ref. 2). Richter4.5 concluded that the outbursts of the latter were essentially similar to those of 12 other comets. The omission of these data is therefore unjustified.

Figure 1 is a histogram of the number of observed cometary outbursts  $N_0$  in the interval R to R+0.25 AU as a function of R, the distance between the comet and the Sun at the time of outburst. Three regions are apparent:

- (a) R < 1 AU. The increase of  $N_0$  with Ris simply a reflection of the distribution of cometary perihelion distances8.
- (b) 1 < R < 5.5 AU. A minimum deviation fit gives

$$N_0 = (24 \pm 4) R^{-(2.2+0.1)}$$

Contrary to ref. 1, it is thought that here  $N_0(R)$  is determined chiefly by observational selection, the ratio between detected and undetected outbursts decreasing as the Earth-comet distance increases.

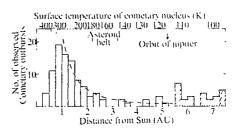


Fig. 1 Number of observed cometary outbursts as a function of distance from the Sun R. Hatched regions, Comet P/Schwassmann-Wachmann (1); White regions, other comets; dashed curve, minimum deviation fit to the data in 1 < R < 5.5 AU region.

(c) R > 5.5 AU. These data originate in toto from comet P/Schwassmann-Wachmann (1) containing outbursts from two complete orbits. There is no clear variation of  $N_0$  with R. It is therefore concluded that the probability of outburst occurrence for the other comets is also independent of R, at least for R < 7.5AU, and similarly independent of temperature for  $T > 115 \,\mathrm{K}$  (the surface temperature of the cometary nucleus is shown as the upper abscissa in Fig. 1, this being calculated assuming that the cometary nucleus has an albedo of 0.6 and is a rapidly rotating sphere). This rules out the three temperature-dependent theories of outburst production—Whitney? requiring T > 130 K to produce pockets of vaporised methane, Donn and Urey8 requiring T > 148 K to produce the explosive NH<sub>4</sub>N<sub>3</sub> and Patashnick et al.<sup>1</sup>

needing T > 140 K for the phase transition between amorphous and cubic ice. This latter theory also requires amorphous ice to have the suspiciously high density of 2.3 g m<sup>-3</sup>, implying an O-O distance of ~ 2.0 Å compared with the more normal 2.8 Å. None of these can account for the outbursts of P/Schwassmann-Wachmann (1).

Three theories remain—tidal disruption by Jupiter, asteroidal collision and collision with boulders9 (mass~108 g). Pittich<sup>2</sup> has ruled out the first two, leaving only the boulders.

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#### Changes in the latitude of the climatic zones of the Northern Hemisphere

In a discussion of the drought in the Sahelian Zone of Africa it has been suggested1 that the drought is associated with a shift of the climatic zones of the Northern Hemisphere towards the equator. An analysis by Lamb was quoted as evidence for this shift, but Lamb's analysis was mainly related to the

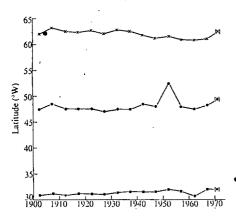


Fig. 1 Average latitudes of climatic zone parameters in the North Atlantic (whole year). Five year means except the last plot which is 1970-1973. ×, Subpolar flow; •, westerly maximum; , subtropical high.

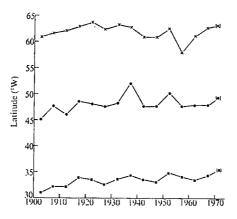


Fig. 2 Average latitudes of climatic zone parameters in the North Atlantic (summer). Five year means except the last plot which is 1970-37.  $\times$ , Subpolar flow; •, westerly maximum; , subtropical high.

subpolar pressure minimum, and the latitude of the subtropical pressure maximum would seem to be more relevant to the Sahelian zone.

Using values of sealevel pressure stored on magnetic tape in the form of a grid, the latitudes of the subpolar minimum of pressure, the subtropical maximum of pressure and the latitude of strongest westerly winds were calculated. Nonoverlapping periods of 5 yr from 1900 to date were used, both for the northern hemisphere and the North Atlantic region (10°W to 60°W). Figure 1 shows these three parameters for the Atlantic region for the whole year and Fig. 2 for the Atlantic for the summer (June, July and August).

The data for the whole year confirm Lamb's analysis in indicating a slight trend of the subpolar pressure minimum towards the equator but show no trend or even a slight one towards the pole for the maximum of the westerlies and the subtropical pressure maximum. The data for summer lead to broadly the same conclusion, except that the trend of the subtropical pressure maximum towards the pole is stronger. The results for the whole hemisphere are similar. What

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is particularly notable is the trend of all the parameters towards the pole for the whole year and for the summer over the past three 5 yr periods while the Sahelian rainfall has been decreasing. This makes the hypothesis that the present Sahelian drought is due to a shift of the climatic zones towards the equator scarcely tenable.

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Received August 22; revised September 23,

<sup>1</sup> Winstanley, D., Nature, 243, 464 (1973).

#### Role of arginase in the epidermis

THE article by Oka and Perry concerning the role of arginase in the mammary gland1 suggests an explanation for the high levels of this enzyme in the skin2,3. This tissue resembles mammary gland in that the remaining enzymes of the urea cycle seem to be absent.

A large body of work exists regarding the stimulation of epidermal proliferation as a result of wounding. Such stimulation is preceded by the induction of ornithine decarboxylase and the production of putrescine and spermidine under the influence of the epidermal growth factor 4.5. Thus, it seems possible that the function of arginase, at least in the epidermis, is to provide a reservoir of ornithine for the production of spermidine. This idea is consistent with the observations that cutaneous lesions associated with pathological proliferation of rete cells, such as psoriasis and the common wart2, or following experimental application of carcinogenic substances, show greatly increased arginase activity.

Of course it is possible, as Oka and Perry point out, that arginase may also be involved in the production of proline, although this seems less likely to be of significance in the epidermis than in the dermis.

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A description of the most important species of mosquitos occurring in the USSR. Introductory part containing data on morphology, biology, epidemology and distribution. Systematic part; keys to adults and larvae and illustrated descriptions, with the stress on characters of diagnostic value. (Translated from the Russian.)

September 1974

412 pages

Published by Israel Program for Scientific Translations Ltd., and distributed by John Wiley and Sons Ltd.

#### ANIMAL CELL CULTURE AND VIROLOGY

Edited by Robert J. Kuchler, Rutgers University.

Papers deal with the role animal cell culture has played in unfolding the details of the biochemistry of the replication of viruses. Brings together the disciplines of cell culture and virology because it is becoming increasingly clear that viral physiology is very closely linked to the physiology of the host cell infected by the virus. Provides background in the biology of cultured cells and current information on structure and composition of viruses. (Benchmark Papers in Microbiology.)

October 1974

480 pages

Published by Dowden, Hutchinson and Ross Inc., and distributed by John Wiley and Sons Ltd.

#### ADVANCES IN ENZYMOLOGY AND RELATED AREAS OF **MOLECULAR BIOLOGY, Volume 41**

Edited by Alton Meister, Cornell University Medical College.

Offers authoritative reviews of the progress in various branches of this expanding science. Authors have made significant contributions in each of the areas covered and emphasizes recent developments.

October 1974

372 pages

£11.35

#### **OBESE HUMANS AND RATS**

By Stanley Schachter, Columbia University and Judith Rodin, Yale University.

Examines the behavioral similarities of obese humans and animals with lesions in the ventromedial hypothalmic nuclei (VMH)-the presumed satiety center in the brain. Proposes a theoretical formulation to incorporate diverse findings on humans and animals, presents tests of the model on humans, and suggests tests for animals. (Complex Human Behaviour Series).

November 1974

192 pages

£6.00

Published by Lawrence Erlbaum Associates, and distributed by John Wiley and Sons Ltd.

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# reviews

# Pavlovian views in Soviet science

Biology and Neurophysiology of the Conditioned Reflex and its Role in Adaptive Behaviour. By P. K. Anokhin. Pp. xvi+574. (Cerebrovisceral and Behavioural Physiology and Conditioned Reflexes.) (Pergamon: Oxford and New York, June 1974.) £17.00.

Few historical figures command the attention of working scientists. Pavlov, however, remains important, not only for the obvious historical reason that he opened up a new and fruitful field of study, but for the eminently practical reason that many of his detailed findings and behavioural analyses are as valid today as when they were first formulated. Among western students of animal learning, interest in classical or Pavlovian conditioning is probably greater today than it has ever been: it has become increasingly clear that such conditioning is of wider scope and greater importance than has been suggested by the traditional American emphasis on instrumental learning and the law of effect.

It is interesting, therefore, to see how the Pavlovian tradition has fared in the Soviet Union, and one may welcome a major statement by one of Pavlov's students, even if blenching at the price one is asked to pay for it. Even more welcome might have been a statement by a psychologist of a younger generation, but it still seems that only those who sat at the feet of the master can hope to have their books translated into English. Can Soviet psychology really be so patriarchal, or is this a reflection on the policy of western publishers?

It must be said at the outset that this is not a book for casual browsing. It is very hard going. A major problem is that, as is here amply confirmed, Soviet and western students of conditioning have taken over quite different facets of Pavlov's work. Pavlov did two things: first, he studied the behavioural facts about the new conditional reflexes he had discovered by accident, and attempted to elucidate the laws governing their acquisition, extinction, generalisation, and so on; second, he attempted to infer the physiological



Ivan Pavlov-still the Soviet patriarch?

mechanisms responsible for these behavioural laws. Although Pavlov did not draw a sharp distinction between these tasks, it is important to recognise that they do indeed differ. Western behaviourists, at any rate, have followed the former programme of research; but in the Soviet Union, where the study of conditioning is described as the physiology of higher nervous activity, it is the latter interest that has prevailed.

Even if it is accepted that the eventual goal of psychology is to understand behaviour in terms of the functioning of the nervous system, it seems likely that this goal will be attained only after the establishment of valid behavioural laws, and the achievement of some insight into the general logic of the system which produces that behaviour. Although the neurophysiological knowledge which Anokhin can draw on is immeasurably more sophisticated than that available to Pavlov 50 years ago, it is not clear that this increase in knowledge has advanced our understanding of conditioning. Anokhin's use of neurophysiological evi-

dence, at any rate, is often distinctly peculiar. A good example is his discussion of the "accursed problem" (Pavlov's description) of inhibition. Behaviourally, the term inhibition is used in several ways: an inhibitory stimulus, for example, is one which signals the omission of the unconditioned stimulus; an inhibited response is one which does not occur in a situation in which it has occurred in the past. In neurophysiology, the term is used in a different set of ways, here most often to refer to the blocking of neural transmission ascribed to postsynaptic hyperpolarisation. Anokhin has no qualms about using this neurophysiological concept to dictate a number of psychological conclusions. Thus, since physiological inhibition "exists only so long as the excitation that gave rise to it exists," inhibitory conditioning can occur only in response to prior excitatory conditioning. Again, since physiological inhibition is a "purely local process" which "always remains at its point of origin," we can reject Pavlov's theory of inhibition which assumed that inhibitory stimuli

themselves acquired new, inhibitory properties, for that requires the assumption that inhibition is initially localised in sensory cortical areas, and would then have to be propagated to the cortical representation of the unconditioned reflex. Indeed, the fact that physiological inhibition does not irradiate over the cortex is used by Anokhin to establish an even stronger conclusion: since the effects of inhibitory conditioning to one stimulus do generalise to similar stimuli, such conditioning cannot, in fact, be inhibitory at all; its effects must be caused by the excitatory conditioning of an antagonistic motivational state.

Most western psychologists, I think, would agree that this type of argument was radically misconceived. What they will find worse, is the way Anokhin continually shifts back and forth between behavioural and physiological evidence, with no clear recognition of the difference between the nature of the evidence in the two cases. With some effort, one can discern the similarities between Anokhin's behavioural arguments and those familiar to western psychologists. Thus, Anokhin's general analysis stresses the importance of a feedback principle (the action acceptor), whereby discrepancies between behaviour and goal are registered and serve to initiate correcting action. Inhibitory learning is then said to arise when the organism detects an adverse discrepancy between the actual and expected outcome of a trial (as the omission of expected food), and this generates an aversive motivational state which suppresses the organised appetitive activity originally conditioned by the presentation of food. There is an obvious resemblance to the version of frustration theory proposed by Spence, Amsel and others, and Anokhin occasionally adduces behavioural evidence in support of his account, which is gratifyingly similar to that advanced in support of frustration theory. But this is Interspersed with neurophysiological evidence, and separated by chapters of neurophysiological analysis, the behavioural implications of which are at best obscure, and at worst patently nonexistent.

As its title suggests, the book is concerned not only with the analysis of conditioning and learning, but also with the biological significance of unconditioned reflexes, and the nature of adaptive systems. Occasional passages in these chapters ring true to a western ear, but it is hard to find many examples of sustained argument or of close interplay between data and theory. It seems depressingly insular to say so, but if there is anything for us to learn from Soviet psychology, it is not apparent from this book.

N. J. Mackintosh

#### Book of the moth

The World of Moths. By Michael Dickens and Eric Storey. Pp. 127. (Osprey: London, 1974.) £2.25.

In a world that contains over 100,000 species, authors who must restrict an introductory book to cover only 100 species have a difficult task. Although there may be a temptation to choose only British species, I am delighted to find that this small, unpretentious book makes those new to the subject, especially young people, immediately aware of what exists in the tropics. It dispels the illusion that moths are "dirty brown things" and although 'professionals' will argue that but a few families are represented, with emphasis only on hawk moths (30 species) and silk moths (39 species), I think this is a delightful 'starter's book' with a whole page of useful information devoted to each species illustrated. The coloured photographs are splendid and I am sure that, at its modest price, the book will be a favourite Christmas gift.

E. R. Laithwaite

# Radiation, ecology, and Soviet concern

Radioecology. Edited by V. M. Klechkovskii, G. G. Polikarpov, and R. M. Aleksakhin. (A Halsted Press Book.) Pp. xii+371. (Wiley: New York and Toronto; Israel Program for Scientific Translations: Jerusalem and London, 1973.) n.p.

RADIOECOLOGY began during the 1920s with studies of the biological effects of ionising radiation on living organisms in regions with high concentrations of natural radionuclides. By the 1950s it had become clear, at least to the Soviet Union, that the wider environmental effects of increasing radiation levels (especially from atmospheric nuclear testing) were so poorly understood that a vastly enhanced research effort was required. This book, written because the "work done by Soviet scientists has been ignored in published material outside the USSR", reveals just how seriously the Soviet Union takes the problem and how extensive their work

The two fundamental problems in radioecology are to determine how radionuclides migrate within biogeological systems and how ionising radiations affect microorganisms, plants and animals. The \*17 chapters describe Soviet researches into the ways that radionuclides (natural and artificial) and radiation behave in soils, forest communities, crops, food chains, natural pastures, wild and farm animals, the far north, water, fish, aquatic plants and man. There is also

a short discussion about the practical problems associated with the measurement of radioactivity in complex natural environments.

Many of these studies have evidently been undertaken with an eye to the consequences of exploiting nuclear energy for peaceful purposes. The editors see nuclear power taking a "prominent place" in the Soviet Union in the near future and envisage the extensive use of nuclear explosions in mining and construction. This being the case, they argue that an understanding of the ecological effects of radioactive pollution must "play an important role in the drafting of suitable restrictions".

Peter J. Smith

# Renewed hope for developmental biology

Experimental Studies of Amphibian Development. By E. Hadorn. Pp. vii + 138. (Springer-Verlag: Berlin, Heidelberg, New York, 1974.) \$8.20.

This short and excellent book is a translation from the German, designed "to introduce the reader to experimental research on development".

Developmental biology is experiencing what Professor Hadorn archly describes as a "Period of Renewed Hope" but many would say that it still remains in a delicate condition. One problem is a growing mass of experimental data that is too flexible and provides unconvincing support for too many theories. Another is that we do not know which organisms to concentrate on. The traditional choice has been the Amphibia, which Hadorn makes the subject of his book. Yet his presentation is far from traditional; his method is to avoid the litigious citation of references and to concentrate on describing a series of clean experiments while omitting the messy

Professor Hadorn guides us through the growth, of an individual with experimental analysis as we go along. He mixes deep descriptions of such knotty problems as gastrulation and induction with the light relief of metamorphosis and the mating habits of newts. The book describes many classical experiments and also includes recent discoveries; for instance, we are told that Millipore filters, long used by deluded embryologists as supposed barriers to cell contact, do not act as barriers at all.

The description and diagrams are clear and the subject matter well chosen. In the past, developmental biology has suffered from heavy presentation, but this book offers a delightful contrast.

Peter Lawrence

## Broad visual scope

The Vertebrate Retina: Principles of Structure and Function. (A Series of Books in Biology.) By R. W. Rodieck. Pp. x+1044. (W. H. Freeman: San Francisco, 1973.) \$39.50.

According to the author this book is an attempt to explain in a simple way, what is known about the organisation and function of the vertebrate retina. That is a formidable task, to which the author devotes over 900 concisely written pages. The coverage is comprehensive, ranging from basic concepts in light and photochemistry, through visual pigments, anatomy and ultrastructure, development, electro-physiology, and behaviour. The result is a real compendium; information and an entry into the literature can be found for topics as diverse as the retinal structure of deep-sea fishes and the characteristics of human colour vision.

It is very unusual to find a book with this breath of cover written by a single individual. The advantages: of having only one author are displayed clearly: the plan is coherent, the style of writing is uniform, and repetitions are avoided. There are, of course, also disadvantages. In particular, the coverage is necessarily uneven since it is difficult or impossible for any one individual to be expert in all the fields that are dealt with. In general, I found the coverage of those topics I know little about impressive and very interesting, whereas in those topics with which I am more familiar the treatment seemed rather oversimplified. The treatment of behavioural work with infraprimates seems particularly inadequate. Colour vision in fishes, for example, is dealt with in six lines, including an endorsement of Walls' statement that the best that can be said is that no fish is known not to have colour vision. In fact, there is a great deal of data on that topic, including determinations of wavelength discrimination and spectral saturation functions, and demonstrations of trichromacy, colour mixture, colour contrast, and constancy. Such data are clearly important in view of the great amount of electrophysiological work in that field. It is possible to single out other topics which receive similarly incomplete treatment, but that would be invidious since the general impression is not of the gaps, but of the striking completeness of the description the book gives of vision at the retinal level.

The book is written in a conservative style, presenting a balanced view of the topics and problems raised. Such speculation as occurs is restrained, and is closely attached to experimental data. The book is therefore probably more suited to readers who already have a

reasonable knowledge of vision than it is to, for example, beginning students. The book is without doubt a valuable addition to the literature, and probably represents the most comprehensive review of the whole field that is available at present. References are included up until 1972, so that some of the material is a little out of date, but that is inevitable in a book devoted to a rapidly developing field.

One unusual feature is the complete translation of Ramon y Cajal's monograph on the retina, originally published in 1893, which is included as an appendix. This is, in fact, the second English translation of this work to appear recently, and we should be grateful that after all these years this fundamental study is becoming readily available to English-speaking readers.

W. R. A. Muntz

#### Cold avian studies

Bird Migrations: Ecological and Physiological Factors. Edited by B. E. Bykhovskii. Pp. v+298. (Halsted, a division of Wiley: New York and Chichester. Israel Program for Scientific Translations: Jerusalem, April 1974.) £12.00.

This book is not, as may be expected, a general textbook, but rather a collection of five papers linked together because the work on which they were based was carried out at Rybachi (formerly Rossitten) in the south-eastern Baltic. Paevskii presents detailed lists of ringing recoveries and species maps, which take up two fifths of the book.

Blyumental considers the development of the autumn migratory state based on physiological examinations of 80,000 birds of 15 species. Lyuleeva discusses the energy requirements of swallows in flight during migration. Two short papers by Dol'nik and Gavrilov are on the calorific equivalent of body weight variations in chaffinches and on energy metabolism during flight in some passerines.

It is certainly very useful for those lacking access to the Soviet literature to have these translations. The only pity is that they have to be so expensive. The hard-back format seems to be an unnecessary luxury.

Many western researchers are unaware of the mass of work on bird migration work that has been going on in the Soviet Union. This collection of papers will open their eyes. An even wider deployment of Soviet workers and funds in this general field is at present under way.

One cannot but have reservations about the methods of Lyuleeva. Apart from allowing birds to starve and die in refrigerators, she devised an experiment of profound nastiness. In order to estimate the amount of energy expended in flight, she caught 30 swallows on their nests, weighed them and released them up to 70 km away. To prevent them taking sustenance on the return journey she sewed their beaks shut. She recaptured and reweighed only 17. Any scientist with a spark of decency will be revolted by such unthinking, useless brutality.

G. V. T. Matthews



Chinese bronze sculpture from the middle Chou period (circa 900-600 BC). The patterns represent hair whorls. From The Husbandry and Health of the Domestic Buffalo. Edited by W. Ross Cockrill. Pp. xiv+993. (Her Majesty's Stationery Office: London, 1974.) £8.00; £20.00.

### Time for plates of turtle

Sea Turtles and the Turtle Industry of the West Indies, Florida and the Gulf of Mexico. Revised edition by Thomas P. Rebel. Pp. 250. (University of Miami: Coral Gables, Florida, March 1974.) \$10.00.

It has often been pointed out that our exploitation of the food resources of the sea remains very largely at the hunting and gathering level. We consume not merely very little of the primary plant production of the sea but very little of the herbivore life too. Mostly, we consume predators further up—sometimes much further up—the food chains, with heavy losses of energy on the way. A book which discusses a, tensively annotated. group that includes important herbivores is, therefore, welcome.

The aim of this book is practical the assemblage of the available information necessary "to the proper development and control of our turtle resources". Dr Rebel starts with the description, identification and distribution of marine turtles, which is accompanied by good figures. Then he reviews what is known of the life history of the six species of turtles found in the western Atlantic and adjacent

#### Television:

With a programme entitled "The greatest advance since the wheel" Horizon (BBC2, November 25, 9.25 p.m.) hit top form yet again. The great advance referred to is superconductivity, which, we were told, is now at the same stage of development as was aviation just before Lindbergh's epic flight. If some similar breakthrough could be achieved with a dramatic development involving superconductivity, then industry would have to recognise the enormous potential offered by developments in the laboratory over the past few years.

Having hit the viewer with this dramatic theme, the programme settled down in slightly more sober vein, and care was taken not to overdramatise achievements already made, nor to underestimate the difficulty of getting industry to provide funds to develop the full potential of superconductivity. As one researcher put it, "you can never convince economists that innovation is worthwhile unless there's a war or crisis". But this innovation does • meed scarcely any power to run, just a seem genuinely worthwhile, though I doubt that it can achieve success in all the diverse areas covered in the programme.

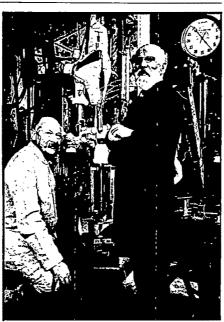
Superconducting magnets are, of course, already in use in powerful accelerators and fusion research experiments. Their advantages are that they

seas; these are all the marine turtles known in the world save a seventh species in Australasian waters. This part of the book contains many scattered pieces of information with gaps in between; separate observations have been recorded in disparate fashions. For none of the species of turtle do we have a complete life history, even in outline, but that is no fault of the author's-it reflects the dearth of sustained and methodical observation.

Turtle fishing and the commercial uses of turtles are reviewed and the author describes the progress which has been made in the cultivation of turtles and makes an assessment of the future prospects. A valuable final feature of the book is a bibliography of nearly a hundred pages covering academic as well as practical matters, which is ex-

Considering the greed with which turtles have been consumed at all stages of their lives with never a thought for the morrow it is astonishing that there is yet time to conserve and exploit them sensibly. Dr Rebel's book gives us reason to believe that turtle populations can be rebuilt and managed, to give a sustainable yield of animal protein and by-products. Let us hope that Dr Rebel will have the reward of seeing this begin to happen in his lifetime.

Garth Underwood



J. D. van der Waals (right) and H. Kamerlingh Ounes-pioneers in the field of superconductivity

few kilowatts to keep the helium in the liquid state, and they don't heat up. We were shown prospects of two really big developments: d.c. motors and a.c. generators.

The former is still looked on with suspicion by industry, but seems to have been clasped to the bosom of the

Royal Navy, which is always interested in the prospect of more power from a motor which weighs less. This conjures up some bizarre visions—"it's no use. Sir, we've been hit in the liquid helium bath"- but no doubt such equipment would be no more vulnerable than the complex electronic computers which are already on many modern ships.

As for generating a.c. power with the aid of superconducting coils, industry, it seems, just doesn't want to know. Power generated in experimental setups is tiny by industrial standards: 1 MW, a fraction of 1% of the output of the standard generators used in power stations. The description of research in this area left me slightly puzzled. It seems that the development is so difficult because the superconducting permanent magnet must be made to rotate (cooling helium and all) inside the generator coils. Why, I wonder, do the experimenters not leave the magnet fixed and rotate the coils around it? There is probably a simple explanation, but the point should have been made clear.

There was also a slight puzzle in the otherwise straightforward explanation of superconductivity. According to Bardeen, Cooper and Schrieffer it seems that electrons travel in pairs through a superconductor, and that this prevents them being scattered by impurities in the metal. Perhaps that is what the mathematics tells us, but the physical description and animation offered by Horizon was too simplistic and perhaps too naive. Someone could have been on hand to offer more elucidation.

The programme ended as it began, with some more flamboyant stuff, this time about superconducting coils used in magnetic levitation trains. But hard core industrialists would probably have been more intrigued by two more straightforward uses of the new wonder, mentioned earlier in the programme. The powerful magnetic fields produced with the aid of superconductivity can be used to separate weakly magnetic ores from rock which would not otherwise be economically workable; and in a similar way suspended colloidal iron oxide can be removed from water, taking with it just about any impurities which may be present. That offers a better way to treat sewage, to scrub the dirty waters of polluted rivers clean, and so on. And there is no pie in the sky about these straightforward applications of superconductivity.

Perhaps not quite "the greatest advance since the wheel"-it would. incidently, be interesting to ask a few people what their candidate for this title would be-but good televised science. And that's something to be grateful for. John Gribbin

# obituary

#### Sir Eric Rideal

SIR ERIC RIDEAL, MBE, FRS, the distinguished physical chemist, died on September 25, 1974. He was 84.

From Trinity Hall, Cambridge, Rideal went to Bonn and obtained his PhD there in 1913. After service in the Munitions Inventions Service, he returned to Cambridge in 1920 as Humphrey Owen Jones Lecturer in Physical Chemistry and ten years later became the first Professor of Colloidal Physics. In 1946 he left to be Director of the Davy-Faraday Research Institute and Fullerian Professor at the Royal Institution. He was appointed to the chair of physical chemistry at King's College, London, in 1950. He retired from this post in 1955 and was elected a Fellow in 1963. After his retirement he continued working at Imperial College, London.

#### H. A. Ferreira

HERCULANO DE AMORIM FERREIRA, the Portuguese climatologist, died on May 18, 1974. He was 78.

Ferreira became a professor at the Faculty of Sciences, Lisbon University in 1930 and taught there until 1965. From 1928 to 1937 he also taught at the Portuguese Military Academy and between 1933 and 1934 was visiting professor at Imperial College, London. He was the director of the Geophysical Institute Infante D. Luis in Lisbon from 1937 to 1964. In 1943 he was charged with the reorganisation of the National Meterological Service, which

he directed until 1965. He was an Under Secretary of State for Education from 1944 to 1946.

#### J. Chamberlain

JOHN CHAMBERLAIN died on October 11, 1974. He was 37.

He took his doctorate from Imperial College, London in 1962 and was appointed that year to the National Physical Laboratory. There, for the rest of his career, he worked on submillimetre wave Fourier Transform spectrometry and refractometry. He made important theoretical and practical contributions to precise measurements and was an authority on the high frequency dielectric properties of materials, including solids, liquids, gases and plasmas.

# **Announcements**

#### **Appointments**

Bernard Isaacs has been appointed to the Hayward Chair of Geriatric Medicine at the University of Birmingham.

H. A. Lee has been appointed to the chair of metabolic medicine at the University of Southampton.

George Edward Mawer has been appointed to the chair of clinical pharmacology at the University of Manchester.

I. C. Whitfield has been appointed to the personal chair of neurocommunications at the University of Birmingham.

#### Awards

Julian G. Edwards has been awarded the 1974 Achievement Award of The Worshipful Company of Scientific Instrument Makers for his development of a Pulsed Energy Laser Moni-

Maurice Ewing has been awarded posthumously the Penrose Medal of The Geological Society of America.

Alfred Edward Ringwood has been awarded the Arthur L. Day Medal of The Geological Society of America.

The Chemical Society is offering an additional award in 1974 which has been sponsored by the British Oxygen Company Limited. The award will be made to the author of the best original paper or papers issued in the past five years (since January 1, 1970) covering the chemistry and/or usage of oxygen. This award is available to men and women of British Nationality, including Commonwealth Citizens or those normally domiciled in the British Isles, and is not limited to Fellows of the Chemical Society.

Nominations and/or applications should be sent to Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN not later than January 31, 1975 and the relevant paper(s) must accompany any such nomination/application.

#### Miscellaneous

The Hollandsche Maatschappij der Wetenschappen (Dutch Society of Sciences) has the intention of publish. ing the correspondence of Professor H. A. Lorentz (1853-1928), who was Secretary to the Society during the last eight years of his life.

The editing committee would therefore greatly appreciate receiving any information from persons and institutions having in their possession or under

their custody letters written by Professor Lorentz, and to receive copies of these letters with the permission for their publication.

Please address all correspondence to: Hollandsche Maatschappij der Wetenschappen (Lorentz Committee), Spaarne 17, Haarlem, The Netherlands.

#### International meeting

January 8, Chemical Properties of Drugs, London (Dr R. A. Webster, Department of Pharmacology, University College, London WC1).

#### Reports and publications

#### Great Britain

Great Britain

Government and the Quality of Life. By Robert Cooke. Pp. 16. (London: Conservative Publications Centre, 32 Smith Square, SW1, 1974.) [189 Information Engineering and Society. By F. J. M. Laver. (The Ninth Maurice Lubbock Memorial Lecture, 1st March, 1974.) Pp. 18. (London: Oxford University Press, 1974.) 30p net. [199 Bulletin of the British Museum (Natural History). Entomology. Supplement No. 22: A Revision of the Old World Genus Zamarada (Lepidoptera: Geometridae). By D. S. Fletcher. Pp. 498 + 123 plates. (London: British Museum (Natural History), 1974.) £35.40.

Advice to Lecturers: An Anthology Taken from the Writings of Michael Faraday and Lawrence Bragg. Edited with an introduction by Sir George Porter, F. R. S., and James Friday. Pp. 18 + 4 plates. (London: Mansell Information/Publishing, Ltd., 1974. Published for The Royal Institution.) 85p; \$1.98. [110 Practical Implications of a Non-Profit Economy. (Proceedings of a Symposium co-sponsored by The Sunday Times and The Science Policy Foundation on 26th June, 1974, at the Royal Festival Hall, London. Edited by Maurice Goldsmith. Pp. 59. (London: Science Policy Foundation, Ltd., 1974.) £1.50. [110 Bulletin of the British Museum (Natural History). Mineralogy. Vol. 2, No. 8: The Naturally Occurring Chromates of Lead. By S. A. Williams. Pp. 377–319. £1.95. Zoology. Vol. 27, No. 4: A Review of Scotoecus Thomas, 1910, (Chiroptera: Vespertilionidae). By J. E. Hill. Pp. 167–188. £1.05. (London: British Museum (Natural History), 1974.)

#### Other countries

Smithsonian Contributions to Zoology, No. 158: Pelagic Studies of Seabirds in the Central and Eastern Pacific Ocean. Edited by Warren B. King. Pp. iv + 277. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) 53.70. [29 Smithsonian Contributions to Zoology, No. 162: Studies of the Subtribe Tachyina (Coleoptera: Carabidae: Bembidiini), Part II: A revision of the New World-Australian Genus Pericompsus LeConte. By Terry L. Erwin. Pp. iv + 96. (Washington, DC: Smithsonian Institution Press, 1974. For Sale by US Government Printing Office.) 65 cents domestic postpaid; 45 cents.

Smithsonain Contributions to Zoology, No. 167:
Studies of Neotropical Caddisfies, XVII: The Genus
Smierldea from North and Central America (Trichoptera: Hydropsychidae) (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office, \$1.50 paper.

Smithsonian Contributions to Botany, No. 15. Leaf
Anatomy and Systematics of New World Velloziaceas.
By Edward S. Ayensu. Pp. v + 125. (Washington, DC:
Smithsonian Institution Press, 1974. For Sale by US
Government Printing Office.) \$2.20 Paper.

United States Department of the Interior: Geological
Survey. Water Supply paper 2017. Improvement of
Trout Streams in Wisconsin by Augmenting Low
Flows With Ground Water. By R. P. Novitzki. (Prepared in cooperation with Wisconsin Department of
Natural Resources. Pp. v + 52. (Washington, DC:
United States Naval Observatory Circular No. 146.
Astronomical Data in Machine Readable Form. By
Solomon Elvolve. Pp. 11. (Washington, DC: US Naval
Observatory, 1974.)

United States Naval Observatory Circular No. 147.
Sunlight, Moonlight and Twilight for Antarctica, 19751977. Pp. 19. (Washington, DC: US Naval Observatory, 1974.)

La Theorie Des Pulsations Internes de la Terre:
Complements. By Georges Dubourdieu. Pp. 47.
(Meudon: Laboratoire de Geologie, Avenue Marcellin
Berthelot, 92-Meudon, France, 1974.)

La Theorie Des Pulsations Internes de la Terre:
Complements. By Georges Dubourdieu. Pp. 47.
(Meudon: Laboratoire de Geologie, Avenue Marcellin
Berthelot, 92-Meudon, France, 1974.)

Comptes Rendus des Travaux du Laboratoire
Carisberg. Vol. 39 No. 18. Toral Morphogenesis in
Tetrahymena Cells Synchronized with One Heat Shock
per Generation. By Howard E. Buhse jun. and Erik
Zeuthen. Pp. 493-305. (Copenhague: Danish Science
Press, Lid., 1974.) 11.50 D.kr each.

Toral States Department of the Interior: Geological
Survey Bulletin No. 1388. Geology of the Istaru Quadrangle, Costa Rica. By Richard D. Krushensky. Pp.

World Health Organization. Equipment for Vector
Control. Pp. 179. (Geneva: WHO; Lon

(Washington, DC: Government Printing Office, 19/4.) \$2.70.
United States Department of The Interior: Geological Survey. Water Supply Paper 2022. Water Availability in Central Wisconsin—An area of Near-Surface Crystalline Rock. By E. A. Bell and M. G. Sherrill. Pp. iv + 32. \$4.25. (paper cover) Water Supply Paper 2006. The Pine-Popple River Basin-Hydrology of a Wild River Area, Northeastern Wisconsin. By Edward Oakes, Stephen J. Field and Lawrence P. Seeger. Pp. iv + 57. \$3.20. (paper). Professional Paper 723. Geology and Ore Deposits of the Rico District, Colorado. By Edwin T. McKnight.

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transition metal chemistry. of the inner ear; work on organorography; elucidating the mechanism drugs used in surgery; developing pola-21. Developing muscle-relaxing

Let Einstein be!' restored the status It did not last: the Devil howling 'Ho! John Collings Squire replied:

20. Alexander Pope wrote it and peare; (k) Owen Seaman.

(i) David Law Proudfit; (j) Shakes-Cowley; (h) Edward Baron Thurlow; (f) Andrew Marvell; (g) Abraham Maine; (d) Byron; (e) Robert Burns; 19. (a) Milton; (b) Charles Churchill; (c) Sir Henry James Sumner

Chile. 18. Three: one in Australia, two in

17. Poldhu, Cornwall.

16. Uri Geller (see Nature, October glucosidase.

15. (a) Acid  $\alpha$ -galactosidase; (b) hexosamidases A and B; (c) acid  $\alpha$ -

(see Nature, October 4). is called Hb Barts hydrops syndrome

semia I which in its homozygous state 14. Gene deletion causes a-thalas-

13. Paul Berg's committee. (c) haemin.

12. (a) Chlorophyll; (b) vitamin B<sub>12</sub>; paintings.

It is used for dating wooden panel 11. Dating wood using growth rings.

catecholamines. neuronal transport mechanism for receptor blockade and inhibition of

10. Imipramine. Direct a-adreno-

coast of Chile, in August 1974. The offending vessel was the Metula. 9. In the Magellan Straits, off the Wegener.

8. Continental drift. Alfred eye. Ernst Mach.

7. The world as viewed from his left Allium cepa.

6. Eat it. It is the common onion,

5. Mr Moon.

4. Queen Victoria.

3. Borodin.

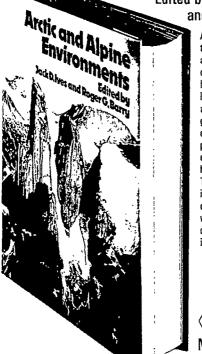
arrangements of leaves on stems. which is commonly found in the from Pisa and 'discovered' the series 2. 89. Leonardo Fibonacci hailed Edinburgh.

1. S. H. Salter, University of (See 980d 992)

Uniz answers

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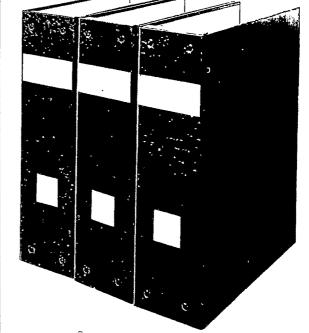
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(2106)

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GENERAL: The Organization's Division of Mathematics and Statistics has over 100 staff members, two thirds of whom are professional scientists located at various regional centres throughout Australia.

The interests of the Chief of the Division, Dr. J. Gani, include applied probability, population processes and epidemic models. The Canberra group currently includes 7 research scientists and is led by Dr. C. C. Heyde, whose main interest is stochastic processes (particularly limit theory). Areas of interest of the other scientists are applied multivariate methods, experimental design, fluid mechanics (including biological aspects), Markov chain modellings, data analysis and genetic—environment inter-action.

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Applications, stating age, education, qualifications, and experience, to Dr T. Graff, Metals Abstracts, The Metals Society, 1 Carlton House Terrace, London SWIY 5DB.

#### **GREATER GLASGOW** HEALTH BOARD

Applications are invited for a TOP GRADE VIROLOGIST to develop the Viral Hepatitis Reference Laboratory of the Regional Virus Laboratory at Ruchill Hospital, Glasgow. The work is associated with the University Department of Infectious Diseases, the Communicable Diseases (Scotland) Unit, and the Blood Transfusion Service. It involves continuing routine service and surveillance as well as research and technical development. Applicants should have experience of human and animal virology. Salary and conditions in accordance with Whitley Council scale for Top Grade Scientists.

Further details can be had from the Secretary,

Further details can be had from the Secretary, Greater Glasgow Health Board, Room 117, 351 Sauchiehall Street, Glasgow, G2 3HT, to whom applications (I copy) should be sent with details of age, qualifications, experience, present appointment, and the names of two referees, not later than December 21, 1974. (2211)

#### UNIVERSITY COLLEGE DUBLIN DEPARTMENT OF BOTANY

Applications are invited for a post on the Academic Staff in the Department of Botany. Preference will be given to a candidate with experience in whole plant physiology.

The current salary scales are in the range:
College Lecturer
Assistant Lecturer
£3,737 to £4,927
£2,590 to £3,535

Entry point on the relevant scale will be in accordance with qualifications and experience.

A non-contributory pension scheme and family allowances are additional to salary. An alternative contributory F.S.S.U. type pension scheme is also

Applications including the names of three referees should be sent to:—
Mr. J. P. MacHale,
Secretary and Bursar,
University College,
Belfield,
Dublin 4.

Latest date for receipt of applications extended to Monday, December 30, 1974. (2209)

### IMPERIAL COLLEGE WATER/POLLUTION **CHEMIST**

Research Assistant required to join multi-disciplinary research team to work on Geochemical aspects of Heavy Metal Pollution. Applicants should preferably have postgraduate or other experience of the chemistry of trace elements in waters and sediments. The appointment will be on the Lecturer scale within the salary range £2,331 to £3,144 including London allowance according to age and experience plus threshold payments. Applications to Professor J. Webb, Applied Geochemistry Research Group, Geology Department, Imperial College, London SW7 2BP.

#### UNIVERSITY OF EDINBURGH DEPARTMENT OF AGRICULTURE

#### LECTURER IN CROP PRODUCTION

Applications are invited for the post of Lecturer, who will be responsible to Professor R. C. F. Macer (Professor of Crop Production) for teaching in aspects of crop production in undergraduate and postgraduate courses, and for the initiation of a research programme in a selected area of crop production.

Applicants should have a degree in Agriculture or Agricultural Science, and postgraduate research experience would be an advantage.

Salary on scale £2,118 to £4,896 plus F.S.S.U.

Applications (giving the names of 2 referees) should be sent to Professor F. W. H. Elsley, The Edinburgh School of Agriculture, West Mains Road Edinburgh EH9 3JG, from whom further particulars may be obtained. The closing date is December 30, 1974. Please quote reference 1043.

Applications are invited for the post of Director at the Royal Society research station on Aldabra Island.

The person appointed will be responsible for the administration of the station and the direction of staff and will supervise a conservation programme concerned mainly with terrestrial but also marine ecology and assist visiting scientists and research students.

Applicants should be at least 25 years old, have a science degree or equivalent qualification, be medically fit and should preferably have expéri-ence of living in isolated conditions, particularly in the tropics.

Salary within the range £2,600 to £3,300 p.a., less a standard charge for subsistence and free transport to and from the atoll will be provided.

The appointment will be for one year in the first instance, with possible renewal for a further eight months. Two months' terminal leave on full salary is granted after one year's service and four months' leave after twenty. Applicants may be accompanied by wives but no children.

Applications with full details of career to date and names of two referees, should be sent to:

The Executive Secretary (L.U.M.), the Royal Society, 6 Carlton House Terrace, London, S.W.1, by January 10, 1975. (2205)

#### UNIVERSITY OF NEWCASTLE UPON TYNE

#### DEPARTMENT OF CHEMICAL ENGINEERING

Applications are invited from suitably qualified persons for appointment as SENIOR RESEARCH OFFICER until July 31, 1976. Candidates should have a good knowledge of the physics of diffusional processes and be interested in joining a team studying mass transfer phenomena in liquidiquid systems. The person appointed will also be expected to undertake limited teaching duties.

Salary will be in the range £2,580 to £4,896 according to age qualifications and experience, plus standard threshold payment of £229.68 p.a. Membership of a University superannuation scheme will be required.

Applications should reach Professor J. D. Thornton, Department of Chemical Engineering, University of Newcastle upon Tyne, not later than January 4, 1975. (2206)

#### TRENT POLYTECHNIC NOTTINGHAM

#### RESEARCH ASSISTANT/ **DEMONSTRATORS**

DEPARTMENT OF PHYSICAL SCIENCES Good honours graduates desirous of working for a higher degree are invited to apply for posts relating to the following projects:-

- (a) Synthesis of pharmacology active hetero-cyclic compounds.

- (b) Synthesis and polymerization of novel monomers derived from monosaccharides.
   (c) Studies on reduced pyrazines.
   (d) Synthesis acid reactions of sulphur heterography.
- (e) Reduced porphyrins.
- Salary £1,250 to £1,350 to £1,450 per annum on a three year contract.

Application form and further details from The Chlef Administrative Officer, Trent Polytechnic, Burton Street, Nottingham, NGI 4BU. (2208)

Department of Health and Social Security British Pharmacopoeia Commission, London

# Chemists/Pharmacists

■ Secretarial and editorial work for British Pharmacopoeia Collection of data from technical journals, foreign pharmacopoeia for reports to committees Assist in work for European Pharmacopoeía.

☐ Degree or equivalent in Pharmacy ☐ Some relevant post-qualifying experience ☐ Age under 30 ☐ Appointment as Higher Scientific Officer (£3100 to over £4000) or Scientific Officer (over £2200 to £3300) according to age and experience \( \subseteq \text{Ref: SB/6/KD.} \)

☐ Application forms (for return by 10 January 1975), from Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke 29222 ext. 500 (or, for 24 hour answering service, London 01-839 1992).

#### Department of the Environment Water Data Unit, Reading

# **Biologist**

■ Investigate relationship between river biota and degree of pollution in controlled waters Advise on biological aspects of the River Pollution Survey of England and Wales.

☐ 1st/2nd hons degree in biology ☐ 4 years' post-graduate experience in field of river biology ☐ Experience of surveying river biota and collecting such data in the field desirable ☐ Age under 32 ☐ Appointment as Senior Scientific Officer (£3350 to over £4650) ☐ Ref: SA/4/HJ.

☐ Application forms (for return by 13 January 1975), from Department of the Environment, Room 446, Lambeth Bridge House, Albert Embankment, London SE1, telephone 01-211 3325 or 7158.

**Procurement Executive, Ministry of Defence** Microbiological Research Establishment, Porton Down

# **Pathologist**

■ Study into pathogenesis, pathology and immunology of infectious diseases (mainly viral) Investigate nature, cause and characteriza-tion of diseases in experimental animals by various routes of administration Work has particular application to vaccine research.

[] Ist/2nd hons degree in medicine or veterinary science or equivalent [] At least 4 years' post-graduate experience in pathology  $\square$  Age under 32  $\square$  Appointment as Senior Scientific Officer (£3350 to over £4650)  $\square$  Ref: SA/5/FQ.

# Virologist

■ Work on safety procedures and practices involving bacteriology and virology Take and assay samples Clinical laboratory work including: serological procedures, identification of pathogens, and blood counting; freeze drying; safety aspects of air filtration, radiation hazards, disinfection and sterilisation Instruct junior staff on safety and hygiene.

☐ FIMLT or degree in relevant scientific subject ☐ Several years' experience in hospital or laboratory practice, and the handling of toxic materials  $\Box$  Age normally under 30  $\Box$  Appointment as Higher Scientific Officer (over £2650 to £3600)  $\Box$  Ref: SA/6/FQ.

 $\square$  Application forms (for return by 9 January 1975), from Microbiological Research Establishment, Porton, Salisbury, Wilts SP4 oJG.



# UNIVERSITY OF THE WEST INDIES JAMAICA

Applications are invited for (a) LECTURESHIP or (b) ASSISTANT LECTURESHIP IN THE DEPARTMENT OF CHEMISTRY. Applicants with interests in Physical Chemistry would be preferred. Salary scales: (a) 136,168 to J\$9,768 p.a. (b) J\$5,006 to J\$5,486 p.a. (£! sterling=J\$2.11). F.S.S.U. Study and Travel Grant. Unfurnished accommodation, for maximum of three years at rental of 10% of salary, thereafter 20% of salary payable in lieu of housing. Family passages. Detailed applications (6 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, as soon as possible to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica. Detailed particulars are available and should be obtained from the same source before an application is made. (2233)

#### CELL BIOLOGIST/BIOCHEMIST

A postdoctoral position is available to work on the mechanisms of connective tissue turnover. The candidate must have a sound background in cell biology/biochemistry but experience in connective tissue research is not essential. The starting salary is £2,412 per annum for someone who has recently completed a PhD training. The appointment is for one year initially, but may be renewed for up to three years, and carries F.S.S.U. benefits.

Please send curriculum vitae and names of two referees to Dr J. J. Reynolds, Tissue Physiology Department, Strangeways Research Laboratory, Worts Causeway, Cambridge, CB1 4RN. (2215)

## ZOOLOGICAL SOCIETY OF LONDON

#### RECORDER

for the **Zoological Record**, a bibliography of the world's zoological literature. Duties include scanning literature and indexing of relevant material.

Applicants should possess a degree in Zoology. Linguistic ability and previous experience in information work would be helpful but not essential. Posts based at units at the British Library Lending Division, Boston Spa and the British Museum (Natural History).

Salary on scale £1,722 to £2,418, plus £228 London Weighting if applicable, plus £229 threshold payment (Weighting under review).

Applications, with brief details, in writing to: Managing Recorder, Zoological Record, P.O. Box 9, Wetherby, Yorkshire, LS23 7EG by January 6, 1975.

(2192)

#### RESEARCH ASSISTANT

To participate in M.R.C. funded project on the role of the neutrophil in allergic glomerular injury. Work in purpose built research block will include human and animal material. Medically qualified person (M.R.C.P. essential) would train in active nephrology department. To start in early 1975. Salary appropriate to grade.

Apply to: Dr P. Naish, Department of Nephrology, North Staffordshire Royal Infirmary, Princes Road, Stoke-on-Trent. (2222)

#### UNIVERSITY OF EDINBURGH DEPARTMENT OF CLINICAL SURGERY RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant to work on the effects of hormones on mammary tumour growth and steroid metabolism in the DMBA treated rat. Applicants should have an honours degree in Biochemistry or a related biological subject. The appointment is suitable for a candidate wishing to study for a higher degree.

Applications, with curriculum vitae and the names and addresses of two referees, should be sent to Professor A. P. M. Forrest, Department of Clinical Surgery, Royal Infirmary, Edinburgh EH3 9YW, from whom further information can be obtained. Please quote reference 5059. (2201)

#### AUSTRALIA

#### PUBLIC SERVICE OF VICTORIA

Ministry for Conservation Fisheries and Wildlife Division

# Senior Research Officer

(Two Positions)

Yearly Salary: \$Al0,505 min. \$Al2,211 max.

#### POSITION No. 1

Ref. No. (N/02)

#### Duties

To carry out field and laboratory studies in marine productivity and energetics in relation to water quality characteristics; other research duties as directed.

#### Qualifications

An approved degree in Science, or other approved equivalent qualifications; appropriate training and experience as related to marine productivity and energetics; experience in experimental studies on productivity an advantage.

#### POSITION No. 2

Ref. No. (N/05)

#### **Duties**:

To carry out investigations into the quantitative ecology of marine systems with a view to assessing the effects of industrial and domestic development of the area; other research duties as directed.

#### Qualifications:

An approved degree in Science, or other approved equivalent qualifications; appropriate training and experience in marine ecology with special emphasis on energetics and productivity; experience with meiofaunal ecology an advantage.

Applications quoting reference number (N/02), should be addressed to the Secretary, Public Service Board of Victoria, State Public Offices, No. 1 Treasury Place, Melbourne, 3002, Australia, by not later than 9.30 a.m. on Tuesday, December 31, 1974, together with statements of experience and qualifications and date and place of birth. (2189)

#### LIVERPOOL SCHOOL OF TROPICAL MEDICINE DEPARTMENT OF TROPICAL MEDICINE **BIOLOGIST/PARASITOLOGIST**

Research Assistant required to work on a programme to investigate action of drugs and drug metabolites on schistosomes maintained in vitro. He will be required to conduct experiments using novel ultrasonic instrumentation, and will work closely with a counterpart in the Bio-Engineering and Medical Physics Unit of the University of Livernool. and Medi Liverpool.

The post is initially for 1 year from February 1, 1975. There is a possibility of registration for a higher degree.

Salary Range: £1,761 to £2,019.

Apply to: Professor H. M. Gilles, Department of Tropical Medicine, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA.

#### M.R.C. CLINICAL RESEARCH CENTRE. (Northwick Park Hospital), Watford Road, Harrow, Middlesex, HA1 3UJ.

Harrow, Middlesex, HAI 3UJ.

TECHNICIAN with H.N.C. or equivalent in Chemistry or Biochemistry to work in a research group studying the biochemistry of inborn errors of metabolism. The work which will be largely concerned with the measurement of amino acids and oligosaccharides in body fluids offers good opportunities of experience in modern methods of automated ion exchange chromatography and gas chromatography. Previous experience with one or other of these techniques an advantage.

Ref. 123D/Z/A23

TECHNICIAN with A.I.M.L.T. or H.N.C. preferably with experience of serology, haematology or bacteriology to work in a laboratory concerned with the study of human immunological disease:

Ref. 118/2/4196

Ref. 118/2/4196

Salary within the range £1,860 to £2,835 plus £126 London Weighting and Threshold.

Application forms may be obtained from Mrs. J. Tucker-Bull. Please quote reference.

#### NATIONAL ORGAN MATCHING AND DISTRIBUTION SERVICE

AND DISTRIBUTION SERVICE

A vacancy exists in the National Organ Matching Service at the Regional Transfusion Centre, Southmead, Bristol, BS10 5ND, for a Science graduate (B.Sc.) with a training in, or good practical experience of computer programming.

The Service makes use of computers in several facets of its work and the appointee will be expected to play a major role in the rationalisation and development of this work. Candidates should therefore have a strong interest in development of information storage and retrieval systems for specialised medical data as well as for scientific analysis, The appointment will be, initially for a two year period and will then be subject to review.

Salary scale, Junior Scientific Officer, commen-

review.

Salary scale, Junior Scientific Officer, commencing at £1,689 rising to a maximum of £2,994.

Application forms and further details of the post may be obtained from the Administrative Officer at the above address. (2199)

#### UNIVERSITY OF OXFORD

Post-doctoral biochemist required to study structure of mammalian chromatin and its relation to genetic activity, financed by Medical Research

Applications and enquiries to Dr. J. M. Barry,
Department of Agricultural Science, University of
Oxford, (2228)

#### M.R.C. CLINICAL RESEARCH CENTRE.

(Northwick Park Hospital), WATFORD ROAD, HARROW, MIDDLESEX, HAI 3UÍ

The Division of Immunology (Head: Dr G. L. Asherson) seeks medical or scientific worker to continue studies on the actiology of immunodeficiency disease in a setting in which clinical and laboratory aspects can be combined.

combined.

Conditions of the appointment, which is superannuable, are similar to those at Universities and the starting salary will depend on experience, age and qualifications.

Application forms may be obtained from Mrs J. Tucker-Bull. Please quote ref. 118/1/4193. (2204)

#### M.R.C. CLINICAL RESEARCH CENTRE.

(Northwick Park Hospital), WATFORD ROAD, HARROW, MIDDLESEX, HA1 3UJ

JUNIOR TECHNICAL OFFICER required in

JUNIOR TECHNICAL OFFICER required in the Division of Communicable Diseases to take part in research on virus, mycoplasma and other infections. Major part of work concerns research on sexually transmitted diseases. Experience in microbiology would be useful. Ref. 108/2/4061.

The Division of Immunology require a JUNIOR TECHNICAL OFFICER/TECHNICAL OFFICER and TECHNICIAN with experience in immuno-chemistry or tissue culture to work with a scientist on immunological problems in humans, Ref. 118/2/A21.

Oualifications: TO: HNC or relevant decree.

Qualifications:- JTO: HNC or relevant degree.
Technician: AIMLT, HNC or relevant degree.

Salary within the range of £1,875 to £2,718 plus Threshold increases.

Application forms may be obtained from Mrs. J. Tucker-Bull. Please quote ref. (2203)

#### RHODES UNIVERSITY GRAHAMSTOWN SOUTH AFRICA

Applications are invited for the post of LECTURER IN PHARMACEUTICS

in the School of Pharmaceutical Sciences from as early a date as possible.

The salary scale is R6,300 by 360 to R9,180 per annum. (Note: £1 sterling=approximately R1.60c).

A vacation savings bonus is payable and the successful applicant will become a member of the University's pension and medical aid schemes.

Applicants should hold a Master's or Ph.D. degree in Pharmaceutics.

Full particulars relating to the post and staff benefits together with application forms, may be obtained from the Registrar, Rhodes University, P.O. Box 94, Grahamstown, 6140, South Africa, to whom completed applications with copies of recent testimonials and a photograph, should be sent by January 18, 1975. (2223)



# **Experimental Officer**

### -Parasitology Project

You would have responsibility for running a unit involved in isolating, identifying and multiplying up a whole range of coccidia in connection with a coccidiosis chemotherapy programme. This work requires critical judgement plus an ability to plan a complex series of operations well ahead, and could suit someone with HNC or similar qualifications. For a more experienced candidate - having a biochemical qualification - there would be ample opportunity of studying biochemical problems associated with drug activity and drug-resistance.

# **Experimental Officer**

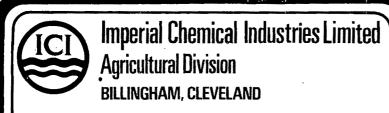
## Obesity Project

This position requires a graduate with a degree of Biochemistry/Physiology to join a laboratory concerned with the identification and development and testing of drugs for use in obesity. A further position exists in this unit for someone of HNC standard to do similar work.

Pharmaceuticals Division has interests in a wide range of human and veterinary medicine and offers modern laboratory facilities set in pleasant parkland, and competitive salaries. The Division is located close to the Peak District and within easy reach of Manchester. A wide range of reasonably priced housing is available in the area.

Please write giving details of age, qualifications and experience and research interests to:

M. F. Losse, Personnel Officer ICI Pharmaceuticals Division Mereside, Alderley Park Macclesfield, Cheshire.



# **Expansion in Biotechnology**

Agricultural Division of Imperial Chemical Industries Limited at Billingham, has an established and successful group engaged in biological research which. since its inception six years ago, has created the ICI Protein Process, an enzyme process and a novel effluent treatment system. The Division now wishes to expand its efforts in biotechnology.

This expansion will involve the creation of additional research project teams concerned with a variety of biological processes. These will include exploratory research directed towards quite new biological ventures, as well as the extension of our single cell protein technology to new proteins and the development of our interests in enzymology. Vacancies exist for

## MICROBIOLOGISTS, MICROBIAL BIOCHEMISTS, MICROBIAL PHYSIOLOGISTS & **BIOCHEMICAL ENGINEERS**

We wish to recruit PhD's and first degree graduates in these disciplines. Successful applicants would be expected to work in multi-disciplinary project teams and be responsible for planning and reporting their own research work.

The Company operates House Purchase, Profit Sharing and Contributory Pension Scheme and offers financial assistance towards removal expenses for married men.

Write, giving brief details of age, qualifications and experience and quoting Reference N/1 to:

Mr M A J W Pegg, Personnel Department, Imperial Chemical Industries Limited, Agricultural Division, PO Box No 1, Billingham, Cleveland TS23 1LB.

(2224)

#### UNIVERSITY OF READING DEPARTMENT OF MICROBIOLOGY

TECHNICIAN required for the Virology section. Duties include preparation of materials for practical classes, assistance to research workers, general supervision of equipment and services and some handling of laboratory animals.

Knowledge of techniques used in Virology and Immunology an advantage. Salary in range £2,247 to £2,628 p.a. (Grade 4).

Apply stating, qualifications, names of two referees, quoting ref: TN100, to Assistant Bursar (Personnel), University of Reading, Whiteknights, Reading RG6 2AH

#### DEAN OF THE FACULTY OF SCIENCE

The newly-established Faculty of Science of the University of Regina requires a dean to consolidate programs and provide leadership. The University is seeking a person with an established reputation in research and with successful administrative experience. The Faculty contains six departments and 70 permanent faculty members. The Faculty offers three and four year undergraduate programs and graduate programs to the Ph.D. level.

Please apply to Mr. D. T. Lowery, University Secretary, University of Regina, Regina, Saskat-chewan, Canada, S4S 0A2. (2195)

#### UNIVERSITY OF ZAMBIA

UNIVERSITY OF ZAMBIA

Applications are invited for 3 posts of READER/SENIOR LECTURER/LECTURER IN DEPARTMENT OF GEOGRAPHY in the School of Education. Candidates must have specialist interests in one or more of the following:—Cultural Geography; Climatology; Quantitative Techniques; Cartography; Air-Photo Interpretation; Regional Planning; Population Geography; Land Resource Analysis. Ability to offer a course in regional geography would be advantageous. Salary scales: Reader K6,800 p.a. K5,600 to K6,600 p.a. K4,000 to K5,400 p.a. (£1 sterling=K1.49). The British Government may supplement salaries of all married appointees in range £516 to £1,026 p.a. (sterling) or supplement salaries of single appointees to the levels of Schior Lecturer and Reader in range £78 to £126 p.a. (sterling) (normally free of all tax) and provide children's education allowances and holiday visit passages. This supplementation is unlikely to be provided to single appointees to Lecturer level. Family passages; various allowances; superannuation and medical aid schemes; regular overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, not later than January 10, 1975 to the Registrar, University of Zambia, P.O. Box 2379, Lusaka, Zambia. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London, WIP ODT. Further particulars may be obtained from either address.

#### UNIVERSITY OF CAMBRIDGE INSTITUTE OF ASTRONOMY THEORETICAL ASTRONOMY

Applications are invited for three research positions at the Institute of Astronomy, which will be available for two years, commencing October 1975. Appointments will be made on the appropriate university scale, currently £2,580 to £3,636 plus threshold payments plus F.S.S.U. benefits, to be reviewed by October 1975. The posts are at post-doctoral level for research in any aspects of theoretical astronomy, and are supported by an S.R.C. grant to Professor Martin

Candidates should apply by letter, giving a full curriculum vitae and the names of two referees. Applications should be sent to The Secretary, Institute of Astronomy, Madingley Road, Cambridge CB3 0HA to arrive by February 15, 1975.

TRANSLATORS (from Russian) and TRANSLA-TION EDITORS, freelance, Control, metering, Lasers, Holography wanted. Box.

#### LOTHIAN HEALTH BOARD

#### METABOLIC UNIT— Western General Hospital, Edinburgh

Applications are invited for the appointment of a SENIOR GRADE BIOCHEMIST in the above Unit which is concerned with the management of and research into endocrine and metabolic disorders. Previous experience in the subject is necessary. Suitable candidates may register for a higher degree and may take part in the M.C.B. training programme run by the Departments of Clinical Chemistry. Salary will be on the scale £3,345 to £4,377. Threshold agreement in operation. Whitley Council conditions of service apply.

Applications, quoting the names and addresses of two referees, should be lodged not later than January 25, 1975 with the Secretary, Lothian Health Board, 11 Drumsheugh Gar-Health Board, 11 Drumsheugh Gardens, Edinburgh, EH3 7QQ. Further particulars may be obtained, or a visit arranged, by contacting Professor J. A. Strong, University Department of Medicine, Western General Hospital, Crewe Road, Edinburgh, EH4 2XU. Telephone: 031-332 2525 Ext. (2173) (2173)

#### UNIVERSITY OF STIRLING DEPARTMENT OF BIOLOGY

#### CHAIR OF ANIMAL BIOLOGY

The University of Stirling invites applications for the Chair of Animal Biology which falls vacant on January 1, 1975. Applicants should be active experimental zoologists working with whole organisms and preferably with an interest in aquatic studies.

Applications together with the names of three referees, should be sent before January 20, 1975 to the Secretary (Na), University of Stirling FK9 4LA from whom further particulars may be obtained.

#### MOUNT ALLISON UNIVERSITY Sackville, N. B., Canada

invites applications for the position of Professor or Associate Professor in its Biology Department effective July 1, 1975. Ph.D. and/or established reputation preferably in Physiology and/or Ecology required. Some administrative experience is desirable. The appointee will be involved in under-

graduate teaching and research.

Apply to Professor D. S. Fensom, Head, Department of Biology, Mount Allison University, Sackville, N.B., Canada. (2190)

#### DEPARTMENT OF PHYSICS UNIVERSITY OF ALBERTA ELECTRONMICROSCOPIST

Applications are invited for an Assistant Professor in the area of Electronmicroscopist. Exceptional candidates in other areas of condensed state physics will be considered. The condensed state group at the University of Alberta consists of eight members in a Physics Department of forty-two faculty members.

The effective date of appointment is April 1, 1975. The closing date for applications is February 1, 1975.

Send vitage list of publications

1, 1975.
Send vitae, list of publications, and names of three referees to:
Dr J. T. Sample
Chairman Department of Physics
University of Alberta
Edmonton, Alberta, T6G 2E1 (X1911)

#### UNIVERSITY OF ALBERTA FACULTY OF SCIENCE DEPARTMENT OF LINGUISTICS

Applications are invited for an Assistant Professorship in Experimental Phonetics, commencing July 1, 1975. Salary range \$13,440 to \$17,611 for 1974-75.

The department's terms of reference are to conduct research and teach in the field of experimental linguistics with some responsibility for university service teaching in general linguistics. Preference will be given to a candidate with special research interests in the physiology of speech perception or speech production with ability to teach service courses in general linguistics. The Ph.D. is essential.

For further information, write to Professor C. I. J. M. Stuart, Head, Department of Linguistics, University of Alberta, Edmonton, Alberta, Canada T6G 2H1. (2180)

#### UNIVERSITY OF THE WEST INDIES TRINIDAD

TRINIDAD

Applications are invited for (a) LECTURESHIP or (b) ASSISTANT LECTURESHIP IN INORGANIC CHEMISTRY in the Department of Chemistry. An interest in modern analytical chemistry would be an additional qualification. Salary scales: (a) TT\$13,200 to TT\$20,904 p.a. Assistant Lecturer TT\$10,716 to TT\$11,748 p.a. (£1 sterling=TT\$4.8). F.S.S.U. Unfurnished accommodation for maximum of three years at rental of 10% of salary, thereafter 20% of salary payable in lieu of housing. Family passages. Detailed applications (6 copies), including a curriculum vitae and naming 3 referees, should be sent by air mail, as soon as possible, to the Secretary, University of the West Indies, St. Augustine, Trinidad. Further particulars will be sent to all applicants. (2232)

#### BIOLOGIST

Graduate required to assist on Genetics Abstracts. The position would involve indexing, proof-reading and abstracting. Starting salary £1,770 p.a. Applications to Dr. E. S. Krudy, Information Retrieval Limited, 1 Falconberg Court, London W1V 5FG. Quote Ref. G1. G1. (2229)

# Opportunities **Overseas**

## Mexico

#### AGRICULTURAL CHEMIST

To conduct a post-graduate course on plant biochemistry with emphasis on agricultural applications and to initiate and supervise research programmes for post-graduate, and final year under-graduate, students. Applicants must possess a degree in Chemistry, Agricultural Chemistry or Agriculture with at least 3 years teaching experience and some experience of research in the field of agricultural chemistry. Appointment for 1 to 2 years initially.

Salary in scale £3,500 to £6,000 p.a. plus a tax free overseas allowance in scale £430 to £1,115 p.a. Other benefits include free family passages, paid leave, children's education allowances, and free accommodation and medical attention. Superannuation rights may be preserved and all emoluments are paid by the British Government. Applicants should normally be citizens of, and permanently resident in, the United Kingdom.

For full details and an application form please apply giving age and brief details of qualifications and experience to :

Appointments Officer

#### Ministry of Overseas Development

Room 301 Eland House Stag Place London SWIE 5DH

(2197)

#### FELLOWSHIPS AND STUDENTSHIPS

#### DEPARTMENT OF EDUCATION **DUBLIN**

#### POSTDOCTORAL RESEARCH **FELLOWSHIPS**

Applications are invited for up to twelve Research Fellowships in Science (excluding Medical and Social Science) and Engineering tenable in any one of the following institutions in Ireland:—

University College, Dublin
University College, Cork
University College, Galway
Trinity College, Dublin
An Foras Talúntais
(The Agricultural Institute),
The Institute for Industrial
Research and Standards.

Research and Standards.

The Fellowships will be tenable for two years with the possibility of extension for a third year.

Applicants should hold a Ph.D. degree or have equivalent research experience or, in the case of engineers or technologists, suitable industrial experience. Candidates should not be over 30 years of age; preferably they should be under 28e

Appointments will be effective not earlier than October 1, 1975. Salary will normally be £2,269 to £2,597 per annum; awards up to £3,061 may be made to engineers and technologists whose industrial experience or other special circumstances warrant!

Full particulars may be obtained from the Secretary, Department of Education (Headquarters Section), Marlboro' Street, Dublin 1. The latest date for receipt by the Department of completed application forms is February 10, 1975. (2174)

#### UNIVERSITY OF ADELAIDE POSTDOCTORAL RESEARCH **FELLOWSHIPS**

The University will award a limited number of Postdoctoral Research Fellowships in 1975. Vacancies exist in most of the Humanities, Social Sciences, Medical and Dental Departments; in the Departments of Agricultural Biochemistry and Soil Science, Animal Physiology, Biochemistry, Electrical Engineering, Architecture and Town Planning, and Statistics; and for research under the direction of Professor J. P. Quirk, F.A.A., Director of the Waite Agricultural Research Institute on the surface chemistry of oxides or pore sizes in gels in relation to soil structure

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THE NORMAL AGE LIMIT is 30 years, and preference will be given to applicants who have qualified for the degree of Doctor of Philosophy or its equivalent within the last three years either elsewhere in Australia or in an overseas country. Tenure will be for two years, with the possibility of extension for a third year.

SALARY SCALE: \$A7,545 to 7,837 to 8,129 to 8,420 to 8,711 (No superannuation provision), Particulars of provisions for travel to and from Adelaide, and other conditions of appointment, are available on request.

APPLICATIONS in triplicate must be made on the special forms obtainable, with the conditions of appointment, from the Registrar, The University of Adelaide, G.P.O. Box 498, Adelaide, South Australia 5001. Applications must be lodged with the Registrar by January 31, 1975. (2193)

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Applications including full name, address, date of birth and details of war service, if any, for the position set out above, marked confidential and addressed to: The Director of Mines, Mines Department, 169 Rundle. Street. P.O. Box 38, Rundle Street Post Office, Adelaide, 5000 (Telephone 223 0461) will be received until and including January 10, 1975.

#### FELLOWSHIPS AND **STUDENTSHIPS**

#### UNIVERSITY OF STRATHCLYDE

Applications are invited for an S.R.C. POST-DOCTORAL FELLOW in the Artificial Organs Division of the BIOENGINEERING UNIT to work on the development of artificial lungs which produce convective fluid mixing for gas transfer augmentation. Applicants should have a Pure or Applied Physics, Chemical or Mechanical Engineering or Chemical Technology background. The studies will involve the analysis and evaluation of problems in gas dynamics, mass transfer and blood gas measurements. Experience in any of these areas would be advantageous.

these areas would be advantageous.

Appointment for three years commencing as soon as possible. Starting salary £2,580 per annum plus threshold payments. P.S.S.U. benefits.

Applications (quoting R43/74) including curriculum vitae and names of two referees to Professor J. P. Paul, Bioengineering Unit, Wolfson Centre, University of Strathclyde, 106 Rottenrow, Glasgow, G4 0NW. (2198)

#### THE UNIVERSITY OF MANCHESTER POSTDOCTORAL RESEARCH FELLOW IN OCCUPATIONAL HEALTH

Applications invited for a Research Fellowship Applications invited for a Research Fellowship from microbiologists to work on the aetiology of byssinosis. Appointment for three years. Initial salary up to £2,300 p.a. The work will involve travel to the Lancashire cotton mills. Further particulars and application forms (returnable by January 3, 1975) from the Registrar, The University, Manchester M13 9PL. Quote ref: 250/74/N.

(2217)

#### UNIVERSITY OF SUSSEX RESEARCH FELLOW

HISTORY AND SOCIAL STUDIES OF SCIENCE

OF SCIENCE

Applications are invited from graduates interested in the social history of modern science preferably with experience of archival research, for the position of Research Fellow in this Division. The Fellow will be appointed specifically to organize, catalogue and list the collected papers of J. G. Crowther, the science journalist. The work will involve materials concerning scientific journalism, science and government, and the "social relations of science" since 1918. The successful Fellow will commence work as soon as possible. The appointment will be renewable for a second year, on the scale £2,118 to £3,285 plus Threshold and F.S.S.U. benefits.

Application forms are available from the Secretary of Science, Science Office, University of Sussex, Falmer, BRIGHTON, Sussex to whom applications should be submitted no later than January 15, 1975. (2230)

#### WELSH NATIONAL SCHOOL OF MEDICINE (University of Wales)

K.R.U.F. INSTITUTE OF RENAL DISEASE, ROYAL INFIRMARY, CARDIFF.

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A graduate in organic chemistry is required to participate in an ongoing study of the metabolism of immunosuppressive drugs in patients with kidney transplants. The successful applicant may be eligible to register for the Ph.D. Degree.

be eligible to register for the Ph.D. Degree.

Further particulars available from The Registrar Welsh National School of Medicine, Heath Park, Cardiff, CF4 4XN to whom application (in the form of a curriculum vitae with the names of two referees) should be made by December 31, 1974. (2200)

UNIVERSITY OF LEICESTER HISTORY AND SOCIAL RELATIONS OF SCIENCE

#### RESEARCH IN INFORMATION **STUDIES**

Applications are invited for participation in work, leading to a Ph.D., on the information content of research papers and their bibliographical control. Applicants should have, or should expect to obtain in the current academic session, a good degree in some area of science.

The Department of Education and Science is prepared this year to offer to suitable candidates a limited number of research studentships on a competitive basis. The value of these awards will be £880 p.a., commencing from October 1, 1975.

Prospective applicants should write for further details to Professor A. J. Meadows, University of Leicester, Leicester, LE1 7RH. (2207)

#### UNIVERSITY OF ABERDEEN UNIT FOR RESEARCH ON ADDICTIVE DRUGS

#### POSTDOCTORAL FELLOWSHIP

Applications are invited for above post supported by the Medical Research Council. Appointment is for two years with possible extension for third year. Salary in range £2,247 to £2,580 plus threshold payments. threshold payments.

Applicants who hold a Ph.D. in Organic Chemistry or Biochemistry are expected to take part in work in progress in the Unit concerned with the identification and synthesis of the endogenous compound with morphine-like actions recently discovered in the central nervous system. Facilities will be provided in the Unit and in the Department of Chemistry.

Further particulars from the Secretary, The University, Aberdeen with whom applications (2 copies) should be lodged by December 22, 1974. (2096)

#### THE UNIVERSITY OF **ADELAIDE**

WAITE AGRICULTURAL RESEARCH INSTITUTE

Applications are invited for appointment from the beginning of 1975 as a

#### POSTDOCTORAL FELLOW (ORGANIC CHEMIST)

in the Department of Plant Pathology. The appointment is funded from an Australian Research Grants Committee grant for up to three years.

SALARY SCALE: \$A7,545 to 292 (2) to 291 (2) to 8,711. Initial salary will be fixed in relation to qualifications and experience. No provision is made for superannuation.

FURTHER INFORMATION is available from the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WCIH 0PF.

APPLICATIONS should reach the Registrar, G.P.O. Box 498, Adelaide, South Australia 5001, as soon as possible. (2181)

#### **GUY'S HOSPITAL** MEDICAL SCHOOL

#### Biochemistry and **Chemistry Department**

Applications are invited for an A.R.C. STUDENTSHIP

for a project involving the isolation properties of a novel steroidtransforming enzyme occurring in ani-mal testis. Applicants should have a good Honours degree in Biochemistry and will be expected to register for a Applications, including curriculum vitae and names and addresses of two referees, should be sent to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, from whom further particulars may be obtained. Please quote Ref. B.C.2.

#### FLINDERS UNIVERSITY OF SOUTH AUSTRALIA , POSTDOCTORAL RESEARCH **FELLOWSHIP**

Applications are invited for a Research Fellowship, tenable for three years, in the School of Physical Sciences. This position is supported by the Australian Research Grants Committee for crystallographic studies of fluorite-related com-

Applicants should have a Ph.D. degree in crystallography, and be familiar with crystallographic computing procedures. Experience in structure analysis on non-molecular crystals will be an advantage.

The appointment will be made in the salary range \$A8,184 to \$A12,331 according to qualifications and experience. Some allowance for travel and removal expenses will be made to the successful applicant.

Applications including full personal details of academic record, experience, and publications, together with the names of at least two referees, should be sent by December 31, 1974, to The Registrar, The Flinders University of South Australia, Bedford Park, South Australia 5042, from whom further details may be obtained.

Applicants are requested to arrange for referees reports to be sent directly before the closing date. (2183)

#### UNIVERSITY OF KENT AT CANTERBURY RESEARCH ASSOCIATE/ RESEARCH FELLOW IN BIOCHEMISTRY

post-doctoral or graduate research worker with qualifications in biochemistry or a related subject is required for work on nucleic acid and protein metabolism of malaria parasites and effects on drugs on these processes. A knowledge of protozoological techniques not expected. Appropriate training will be given. The appointment is supported from external funds, and is for one year with possible extension, from January 1, 1975 or as soon as possible thereafter. Salary in the range £1,782 to £2,580 for Research Associate (graduate) or £2,118 to £2,580 for Research Fellow (with Ph.D.) plus threshold payments.

Application forms and particulars from the Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent. Please quote ref. A78/74. Closing date for applications December 31, 1974. (2187)

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woman).

The I.B.M. Research Fellowships are open to applicants who will have a doctorate (or will have submitted a doctoral thesis) by October 1, 1975 but who will not have had more than three years postdoctoral research experience by that date. At least one fellowship will be offered in the biological sciences and at least one in the physical sciences. Scale of stipends: £2,157 to £2,748 (plus threshold payments).

Application forms and further details are

threshold payments).

Application forms and further details are obtainable from the Secretary of Faculties (Ref. No. IBM/4), University Registry, Broad Street, Oxford OX1 3BD. Closing date for receipt of applications: January 14, 1975.

(2188)

#### LECTURES AND COURSES

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"METHODS OF DETECTION OF IMMUNE COMPLEXES" Dr J. Verrier-Jones.

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"THE INDUCTION OF INFLAMMATORY MEDIATORS BY IMMUNE COMPLEXES" Dr B. Kay.

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Applications should reach Professor B. F. C. Clark, Summer School Secretary, Department of Chemistry, Arhus Universitet, Langelandsgade 140, DK 8000 Arhus C, Denmark, as soon as possible (not later than May 15, 1975). They should contain a curriculum vitae, including the applicant's scientific background and interests, and one letter of recommendation.

Further details can be obtained from the above address or other members of the Organizing Com-

Organizing Committee: M. S. Bretscher (U.K.), B. F. C. Clark (Denmark), A. E. Evangelopoulos (Greece) and A. E. Smith (U.K.). (2213)

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Further particulars may be obtained from the Registrar, London School Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT or the Secretary, Royal Postgraduate Medical School, Du Cane Road, London, W.12. (2194)

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Applications should reach J. Tooze, Executive Secretary, European Molecular Biology Organisation, 6900 Heidelberg, Postfach 1022.40, West Germany, before January 31, 1975. They should include a curriculum vitae, and an indication of the applicant's scientific background and current interests. Applicants will be informed whether or not they can be accepted during March 1975. A registration fee of 40 DM will be charged. Accommodation in Hirschhorn (15 DM to 30 DM per night) will be arranged by the Symposium Secretariat but participants will be responsible for their living and travel expenses. A small number of fellowships are available which will be awarded by the Organising Committee to young participants unable to obtain other sources of support. Persons wishing to compete for these few fellowships should so indicate in their letter of application.

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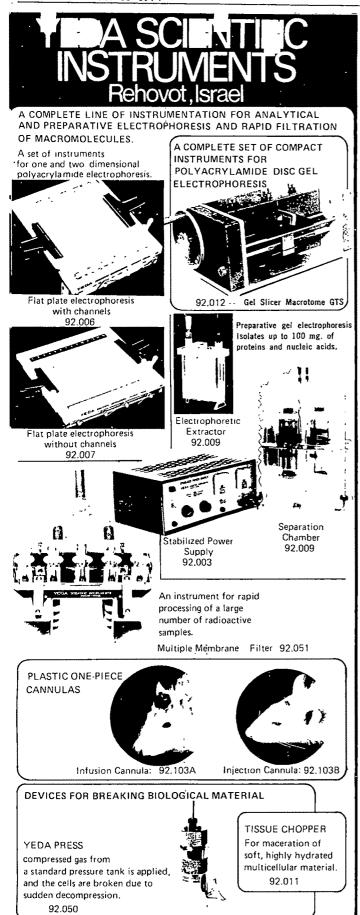
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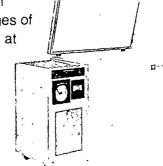
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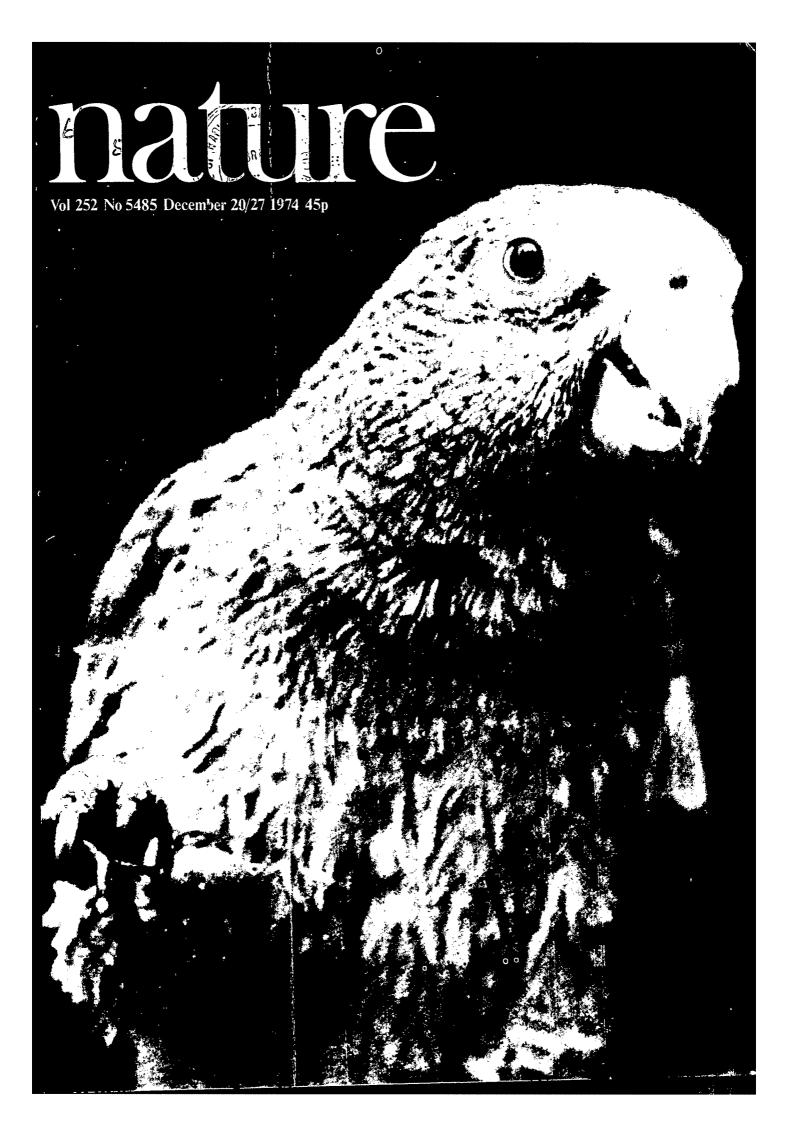
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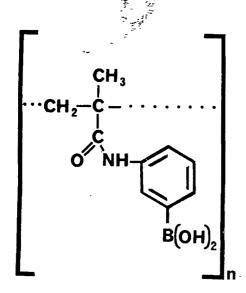
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- 1) H. Schott, Angew. Chem., Intern. Ed. Engl., 11, 824 (1972); [Angew. Chem., 84, 819 (1972).]
- K. Reske and H. Schott, Angew. Chem., Intern. Ed. Engl., 12, 417 (1973); [Angew. Chem., 85, 412 (1973).]
- H. Schott, E. Rudloff, P. Schmidt, R. Roychoudhury and H. Kössel, Biochemistry, 12, 932 (1973).

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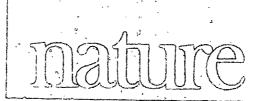
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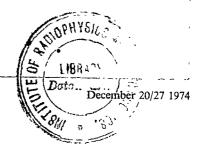
#### Cover Picture

Seraphita, a parrot whose pupils contract when she talks.

See page 637.



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Volume 252

December 20/27, 1974

# Be bold, Mr Varley

MR Eric Varley took over as Britain's Secretary of State for Energy in an almost new government department nine months ago. He had much goodwill behind him as he struggled to absorb the complexities of the nuclear reactor'issues and to negotiate the political and industrial minefield of establishing the government's role in the exploitation of North Sea oil. His decisions on these thorny subjects have been tolerably well received. Alas, while he was coping with the issues of energy in the middle distance, his mind seems to have wandered from the ghastly problems of energy for the present. As a result it has taken nine months for anything of substance to emerge from the Department of Energy on the saving of fuel, and more particularly oil. And what has emerged is modest in the extreme:

- Oil price increases to bear more on the motorist: an extra 8p on a gallon is expected soon.
- Speed limits on roads other than motorways to be reduced.
- Buildings (other than homes) to be maintained at not more than 20° C.
- Improvements in thermal insulation standards for new houses.
- Loans to industry for energy-saving investment—up to a national total of £3 million a year.

None of this is likely to strike terror in the hearts of oil producers. Mr Varley eventually hopes to cut energy consumption by 10% but first estimates are that this package will save only 2%. Drivers on Britain's quaint single carriageway roads would be hard pressed to find stretches where they could exceed the new speed limit of 50 miles an hour. So we must assume that there are other more significant ideas in the pipeline.

The regrettable thing is that it has taken so long to get this short distance. Mr Varley has been gathering around him an Advisory Council on Energy Conservation -a surprisingly lengthy process considering the urgency of the problem—and he has had the benefit of the Think Tank's gratuitous advice this last summer. Yet the measures proposed could have been put together on the back of an envelope the day he arrived in his new office. What is more, nine months ago the mood of the nation was such that tough measures, already imposed in some cases last winter, would have been acceptable as long term propositions. Psychologically, Britain was prepared to live with some austerity. But such constraints as there had been were lifted, presumably for supposed political gain, and by midsummer the man-in-the-street's perception of the energy crisis was that a saving was no longer necessary. Not only the man-in-the-street. A high ranking civil servant in the Department of Energy admitted that the only permanent change in his behaviour as a result of last winter's crisis was that he now turned the light off in his railway compartment when he got off the train. It is



doubtful whether the new measures, which are clearly meant to have as much of a psychological as a physical impact, will restore the lost desire to conserve. What is more, there is almost nothing in Mr Varley's package to encourage industry, the big consumer, to economise.

What is needed is some full blooded government intervention. Whether it takes the form of rationing (and surely the Think Tank could with profit be set to develop a fairly sophisticated scheme for discouraging waste) a massive publicity campaign, jawboning of industrial chiefs or substantial price rises, there are ample opportunities for the government to move fairly deeply into the conservation business. To take one example, such propaganda as the government and nationalised industries have produced so far has seemed unbelievably halfhearted. Discreet advertisements in newspapers will persuade nobody. An enormous effort to raise national consciousness is called for, and this will need a large budget and much imagination. As a result of it, the general public might even end up appreciating solutions of the thermal diffusion equation--more than many scientists do at present. And perhaps someone will develop cheap continuously recording thermometers and other cost-monitoring devices.

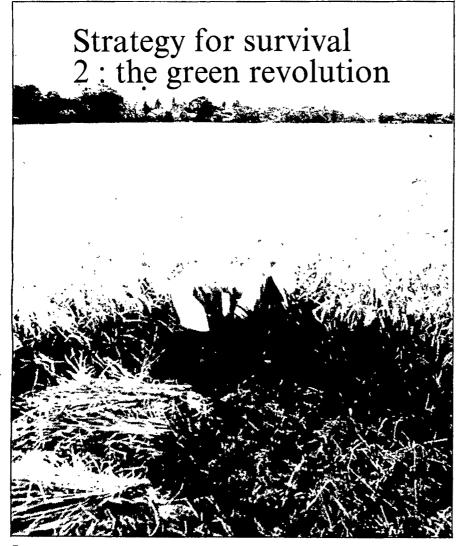
Ultimately, the government will have to face up to the central message of the energy crisis—that our way of life will have to change if Britain is to remain solvent. The same is true, of course, of all other countries similarly afflicted, but Britain will be the first to suffer if radical measures are not taken soon. It is strange that an external military threat would be perceived immediately and the public called upon to make sacrifices, whereas an economic threat is deemed unsuitable material with which to go to the country and ask for belt-tightening. Mr Varley might be pleasantly surprised by the response if he chanced his arm considerably more.

## A hundred years ago



THE process of polishing the lens of the mirror of the great telescope is going on at the French National Observatory by M. Martin. The diameter of the lens is 120 centimetres, and the polisher is a disc of 40 centimetres. The number of men engaged on the polishing is six. They are obliged to stop frequently on account of the great weight of the polisher. An observer placed on the top of the Observatory, at a distance equal to half the focal distance, superintends the polishing process, watching if the image of a light which is placed in a proper position is reflected with sufficient exactness by the mirror below.

From Nature, 11, 154, December 24, 1874.



David Spurgeon looks at a report soon to be published on what the farmers at the grass roots really feel about the Green Revolution.

Although in simple terms of production gains the Green Revolution has been an undoubted success, it has produced a harvest of reproach as well as praise. Critics have maintained that the introduction of the new high yielding varieties of wheat and rice together with the necessary package of technological practices has helped the rich get richer while the poor get poorer. They predicted that it would lead to the mechanisation of farms, reduction of opportunities for labour, concentration of land holdings and other factors which would increase inequities between rich and poor. All this was said without much in the way of factual support.

Now, at last, some real evidence is beginning to come in from the fields and farms of Asia. It appears in the results—soon to be published—of studies of 2,428 rice farms in 36 villages located in 14 separate areas of six Asian countries.

The research, funded by Canada's International Development Research Centre and carried out through the

International Rice Research Institute and a number of Asian institutions, was to determine just what changes had occurred in the wake of the introduction of the new rice technology. Answers were sought to such questions as: How extensively have the new varieties been accepted? Who is benefiting from the new technology, and in what way? How has the new technology affected employment? What have been the effects on social structure? And what are the major obstacles to further growth in rice production?

One major finding, in case anybody was still in doubt, was that the Asian rice farmer is not a stubborn traditionalist thoroughly resistant to change. Certainly he responded to innovation in a way that would minimise his risk, but respond he did: in 29 of 32 irrigated villages, 90% or more of the farmers reported that they had at least tried the modern varieties (which as used here means varieties introduced since 1965). Adoption rates vary from country to country, but in Pakistan,

for example, the area planted with the new rice varieties was 42% of the total only five years after their introduction into that country.

Another important finding was that both family and hired labour increased (and not the reverse, as predicted) in all of the study sites except in two Pakistan villages, where practically no labour changes were reported. This was so despite the adoption of the tractor in several places. Malaysian villages had the highest proportion of those taking on tractors, but pre-harvest labour decreased there nonetheless. In fact, the villages where labour-saving technology had been most widely adopted after introduction of the new varieties also reported the largest number of farmers who had increased their employment of family and hired labour. Apparently any savings in labour were more than offset by the labour requirements of the technology itself. And the introduction of the new varieties seems to have provided more, rather than less, employment to landless farm labourers.

On the question of increased income from higher yields the evidence varies. Income definitely increased for some farmers: in one area of the Philippines, the value of the farmer's harvest in both wet and dry seasons was almost three times what it was before introduction of the new varieties. Costs of production also increased, however, because more fertilisers and pesticides are required for the new varieties. Even then, farmers' incomes were often greater than they had been.

Yet, subjectively, many farmers did not see themselves as any better off. One researcher pointed out that this was partly because farmers and their families had eaten more rice themselves since the introduction of the new varieties while not seeing this as a form of profit, which of course it is. Another suggested that previous losses had left farmers with long-standing debts that could not be entirely offset by a single season's gains. Several researchers reported specific economic gains as a result of the new varieties and detailed how the gains had been spent.

Consumer goods figured high among purchases made by the successful farmers. These included bicycles and radios, sewing machines, house furnishings and farm implements. Many spent more on food and education than they had before, whereas others paid off debts with their increased profits.

Not all farmers felt the same way about their status after the introduction of the new varieties. Many saw themselves as better off but a few even thought they were in a worse situation.

The views of the researchers also differed. One report, from India, seemed to confirm the critics' opinions.

It maintained that "the new technology helped to tighten the grip of the big farmer on rural economy" and that, although there was no firm evidence of deterioration in living standards of small farmers, small tenants and labourers, "relative distribution of incomes appears to have worsened."

Yet in an analysis of the whole picture, Celia T. Castillo, of the University of the Philippines, said: "From the data, it is obvious that tenants and small farms are better off with modern than with local varieties as far as their own assessment of increase in rice profits and level of living is concerned. Of course, owner-operators and large farmers were much better off than tenants and small farmers. How else could it have been? It is truly asking for a miracle to expect that the new seeds would bring about social equality where centuries have failed to produce a dent on institutional rigidities."

Dr Castillo says emphasis on the relative gains made by the rich and poor farmers has led to a neglect of another issue-farmers' gains relative to where they were before the advent of the new technology. If one becomes preoccupied with the income distribution issue, one is left with the impression that tenants and small farmers would have been better off in terms of incomes and living standards if they had stuck with traditional varieties, she says. And if this were the case there would be no rationale for spreading the new seeds more widely. But it is not the case, according to the data.

In another analysis, Randolph Barker and Teresa Anden, of the International Rice Research Institute, state: "It is clear that a technology that requires more cash inputs will tend to reinforce this [income distribution] inequity in some of the study villages. One cannot, of course, expect technological innovations to correct serious inequities in access to and benefits from resources. The only mitigating step that can be taken is to attempt to reduce cash requirements, particularly for chemicals, by building more resistance and tolerance into the seed itself. Increasing emphasis is being given to this problem in rice research.

The necessity for such research was illustrated in both West Java and the Philippines. In the former, about half the wet season crop was destroyed by gall midge, and in the latter tungo virus caused similar damage. Farmers in Java noticed that attacks were more severe on the available new varieties, so they reverted to local ones, while in the Philippines resistant modern varieties were available—and were used.

Barker and Anden conclude, from the responses of farmers to a question about their preferences, that local varieties will remain popular in some areas until modern ones are developed that are suitable to local environmental conditions, or until the strong price preference for local varieties changes. They also note that government policy has been a significant factor in determining the speed with which the new varieties have become available and accepted, if such policy has influenced price and availability.

Dr Castillo pointed to another notable finding of the study: "Although high yield capacity is the characteristic most associated with the new varieties, in many villages where adoption has taken place, their yields did not exceed those of the local varieties; they were adopted because of their shorter growing period and non-photoperiodism." Many farmers used the new varieties because they could provide two crops instead of one. This second crop sometimes replaced one of pulses and vegetables, however, and the impact of this on the protein-poor diet of the farm

family will have to be further analysed.

A clear need emerged for improvement of several factors if the full potential of the new varieties is to be achieved: availability of credit and the necessary infrastructure through which seeds, fentiliser and pesticides can be obtained; irrigation and flood control; and suitable government policies regarding price, information distribution,

and so on.

The study should provide a good factual base from which to consider not only what changes the new rice technology has made in Asia, but also what changes are needed for the future. Those involved in it consider, however, that an equally important result—if not a more important one—will be the strengthening of social science research in the region. In their eyes it is not just another research project; it is a device for developing a network of relationships among Asian social scientists concerned with similar problems.



Land preparation and harvest in the Philippines.



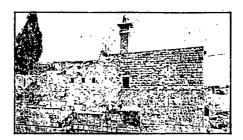
# international news

ISRAEL seems to have adapted the diplomatic 'leak' to suit the requirements of the Middle East confrontation. Appropriately for a nation which so reveres science and the academic tradition, a vague confirmation of the Israeli nuclear capability was made recently, not through a lobby's 'nark' sloping around the back door of the Knesset, but by the President, himself a scientist, in open discussion with a group of visiting science journalists.

Exactly what President Ephraim Katzir, former chief scientific adviser to the government, meant when he said that Israel could build a nuclear device if necessary "within a reasonable period of time" is open to a variety of interpretations. Nobody would be very surprised to learn that Israel had saved enough plutonium from its Frenchbuilt Dimona reactor to manufacture about 10 devices of 20 kilotons-that is, big enough to do to Cairo and Amman what was done to Nagasaki. Neither would it be big news to hear that the aviation industry was working on a delivery system and that, meanwhile, the odd bomber has been set aside for the job. (It's unnerving how many times a day you're told that a Volkswagen roof-rack or a fastish camel might serve as a last resort.)

Anyhow, given the speed with which recent Middle East wars have run their course, "within a reasonable period of time" is more likely to mean "we made a batch of components last year" than "we could manage from scratch in a couple of weeks". This much was already taken for granted.

The only real news was that it was Katzir who was laying it on the line and the following day, when journalists tried to telephone the tidings to editors on the continents of Europe and America, the censor tended to cut in on the call and impose a moratorium. The effect of this kind of reaction is. of course, counter-productive, because jaded editors pay rapt attention to stories which other people want to keep quiet, particularly when the other people are military censors. So when the good news was brought (as inevitably it would be from a purposebuilt junket for professional scribblers) it received an arguably inflated Press statement. Obligingly, an official clarification followed the next day, to the effect that what the President had meant to do was to stress the official line on nuclear arms. Katzir's office said he had been referring "to the general potential in Israel of scientists and general scientific-technological experience that, objectively, could be implemented if Israel so desired". The statement added that the President had simply "reiterated past pronouncements to the effect that Israel will not be the first to introduce nuclear weapons to the area".



Temple Mount, Jerusalem

# Problems in Israel

by John Hall and Peter Newmark

At this point interpretations diverge. The conspiracy theorists say that having gathered a fair sample of Western journalists who were searching for military implications in every science project they were shown, the titular head of state and founder of the country's major defence research centre is scarcely going to blurt out new information about the biggest state secret he knows. Neither is he the sort of character who needs to have his statements toned down by nervy 'official clarifications'. In other words it was all a fix. Katzir knew very well how his remarks would be read and the official correction was sufficiently ham-fisted to make no difference to the general interpretation that Israel was all set to go nuclear. The intervention of the censor, on this analysis, was all part of the act, just to make sure the story got page space.

The point of the exercise, on this •reading, was to spell out plainly to the Arab world that Israel's nuclear-bluff strategy was over at last. The Hebrew University's Middle East conflict scenario studies have shown that the country's intentionally ambiguous nuclear weapons policy has backfired. The idea was that if nobody really knew whether or not Israel had a bomb everybody would be cautious about attacking. You had one guess on the issue and you couldn't afford a fifty-fifty chance of error. In fact, according to the studies, the effect of the 'ambiguous' policy has been to increase the race by other Middle East countries to acquire nuclear weapons. There have also been two wars along the way. So the set-up was intended to say to the Arabs: "No more mystery. We'll use bombs if we need to", while at the same time avoiding international censure by broadcasting a softer official line.

The second interpretation? President Katzir, who was speaking freely and informally, didn't anticipate that every word he said would be subjected to a semantic analysis which allowed a newsstarved reporter to read in an unexceptional remark the story he'd been looking for all week.

• It is traditional for a journalist visiting a foreign land to draw his truisms from the mouths of anonymous taxi drivers, who encapsulate the wisdom of the ages and folkloric charm in a single aphorism which also happens to contain the kernel of the hack's thesis. As if to preserve one's fondest illusions our Rehovot driver settled behind the wheel and volunteered the information that the trouble with scientific research in Israel was that it contained too many chiefs and too few indians. Seventeen per cent of the working population of one million people were academics, and if some of the chiefs left their campuses and became indians in industry, then things would be so much the better, he added, warming to his subject. (Anyone wishing to establish bona fides is referred to Michael Fox, 3a David Shimoni Street, Rehovot.)

In confirmation of Mr Fox's assessment, Dr Eliezer Tal, Director of the National Council for Research and Development, also regretted that the country's research effort was top-heavy on basic work carried out in the universities, and getting more so with every year's intake of immigrants. (An average year's 35,000 arrivals include 350 scientists, and an influx of 600 scientists is expected from the Soviet-American deal to release Russian Jews.)

Research and development spending in Israel breaks down as follows: military, 45% of the total; university research (mainly basic) 30%; industrial, 12%; government laboratory work,

10%; others, 3%. Broadly, says Tal, the three main objectives of the government are to secure a better spin-off from the military work which consumes such a vast proportion of the budget, to look for ways in which the excessive amount of university research can be used to support industrial projects, and to encourage industry to buck up and get on with its own research programmes. To this end incentive programmes are under way which weigh the grant distribution system heavily in favour of the researcher who opts for applied work. Tal points to countries like Switzerland and Sweden as examples of top level industrial producers whose excellence reflects their disproportionately high spending on industrial research. It must be remarked, however, that they have thoroughly complacent taxi drivers.

• In a country whose development depends as much on water as on anything else, it is not surprising that the discovery of new sources is followed by ambitious plans. After the war of 1967, and following a combination of biblical clues and intelligence reports, a search for underground water in the occupied Sinai desert led to the discovery of a considerable reservoir. Carbon dating studies show that this water originated 20,000-30,000 years ago and is therefore unreplenishable fossil water. But according to Arie Issar, a hydrologist at the Ben-Gurion University of the Negev, sufficient water can be tapped to support the equivalent of 60 new settlements for 50 years. More precisely, he estimates that the output could be 50 million cubic metres a year.

The Desert Research Institute of the Ben-Gurion University, set up in 1973, has a substantial interest in this new water source. Situated at Sde Boker in the Negev Desert, 30 miles south of the main university campus in Beersheba, its scientists are undertaking a wide programme of desert research. They hope to be able to use the water to irrigate large areas of the Negev so that agricultural development can take

A combination of basic and applied research has been designed to tackle the problems that this project will have to solve. Apart from the general problems of establishing settlements in the desert and of making life bearable, or preferably attractive, for the settlers, there are more specific questions raised by the quality of the water. It has been found to be both warm (42° C) and somewhat brackish. The institute's scientists claim that the warmth may actually be an advantage when it comes to growing winter crops. The salinity is also unlikely to pose much problem since there are many crops that can cope with the salt levels involved. In addition it is hoped that a programme of plant breeding will yield other and better salt-resistant strains.

Another approach to persuading the desert to support more life than a few Bedouins is that of introducing alien vegetation which will grow even without irrigation. Two promising cases are jejoba, an American desert tree whose nuts are a source of high quality wax, and atriplex, an Australian fodder crop for grazing animals. Once the Negev is tamed, the planners see no end of exciting possibilities. There are promises, for example, of a series of safari parks teaming with African wild life. But right now it is not altogether clear when the promised water will flow into the area from the reservoirs.

• The decision of UNESCO to cut off aid to Israel was met with both sadness and bitterness in that country. Israel's president, Ephraim Katzir, felt that it was "the beginning of the destruction of civilised organisations" and expressed sorrow that politically motivated decisions, which were only to be expected in the UN itself had found their way into UNESCO affairs. To which sentiment the Director-General of UNESCO, Amadou Mbow, effectively replied in a statement that "the delegates to the general conference are government representatives. It is natural that the problems which perturb the world today should find an echo there".

In other words the oil monopoly and the political intricacies of the Middle East are justification for bringing sanctions to bear against the country which has done the most in that area in recent times for education, science and culture. For the alleged claim against Israel, that of endangering and disfiguring religious sites in the course of archaeological excavations in occupied Jerusalem, can not be taken very seriously. It is certainly a difficult job carrying out excavations in Jerusalem where every inch of land seems to be vied for by two or more religions, nowhere more so that at the disputed site outside the Western (Wailing) and Southern Walls of the Temple Mount. There is, however, next to no expert condemnation of the excavations so far undertaken there by the Archaeology Department of the Hebrew University, Jerusalem.

Ironically Israel has actually benefited financially from the UNESCO decision—the UNESCO contribution to the costs of the excavations was only a quarter of the sum paid into UNESCO funds by Israel. And in terms of participation in UNESCO, the position seems to be unchanged, in that Israel's request to join the organisation's European regional group has not been granted. Israelis are disappointed at the lack of reaction from the scientific community abroad but grimly resigned to their increasing isolation.

#### Universities in the not-so-red

British universities have been spared some of the ravages of inflation by the decision of Mr Prentice, Secretary of State for Education and Science, to mentation of the grant for 1974-75 to accepting students for 1975. about £21 million, roughly what the universities had expected for that year on the basis of the rise in costs during the quinquennium remains to be seen. 1973, which is how the government does its calculations.

gone up since then, so, although the future would have to take into account Chancellors and Principals (CVCP) increasing.

welcomed the announcement, as a help to the universities in their "grave" situation arising from the high continuing rate of inflation, they warn that substantial economies will continue.

The Secretary of State will soon be able to announce the level of grant for the financial years 1975-76 and 1976increase their recurrent grant by £15 77. Universities must know this decision million. This brings the total supple- soon say the CVCP as they are already

> What will actually happen to the grants for the remaining two years of

A warning note was struck by Mr Prentice in his announcement when he Of course the rate of inflation has said that the level of grants in the hard-pressed Committee of Vice- the fact that student numbers were not

#### Visa for Voronel

Aleksandr Voronel, the Soviet physicist has received an exit visa for Israel.

In spite of official harassment, illicit Sunday seminars of scientists dismissed after applying for visas for Israel continued meeting during the Autumn in Moscow. On November 15, Voronel was told by the KGB that he could have permission to emigrate provided the seminars were discontinued. Voronel replied that he did not see the connection between his leaving the country and the seminars, which would stop only if the whole group were allowed to emigrate. Nevertheless even though the seminars had been continued in the interim, on December 11 Voronel received an exit visa valid until December 24. 

#### Scandinavian diary

from Wendy Barnaby

but when it comes to cancers the ing the surounding tissues. Focusing Swedes are approaching the problem is done with the aid of a collimator from both angles. January 1, 1975 will helmet arangement, and is amazingly see the introduction of two new mea- accurate. Deep-lying tumours and those sures in Sweden, each aimed at reduc- just under the pituitary body will be ing the incidence of cancers and particularly susceptible to such treatrelated illnesses.

• A revision of maximum allowable concentrations of chemicals in the working environment has recently been of Parkinson's disease may also find published by the National Swedish relief. During some operations the Board of Occupational Safety and Health. Issued against the background be able to communicate with the of reports that various workers have operator through a built-in two-way contracted illnesses ranging from cancer to giddiness, kidney malfunctions, loss of memory and cramps after work
European Space Research Organisa
European Space Research Organisa
Space Research Organisaing for sustained periods with polyvinyl chloride and a jet fuel (MC77), the new law lists 125 substances and specifies the maximum allowable concentration of each to which workers may be exposed, averaged out over an eight hour day or a period of fifteen minutes. The permissible levels of about 80 of the substances have been lowered, some drastically, and new chemicals have been put on the list. Vinyl chloride used to be allowed in concentrations up to 20 p.p.m. The new level will be 1 p.p.m., the same as that set recently in the United States.

Other substances affected by the new law include arsenic, 20-30 kg of which is used daily in the making of glass and for which concentrations will be lowered from 0.5-0.05 mg per m<sup>3</sup>, and chromium salts. Used in the tanning of leather and as pigments in anti-rust paints, these will in future be allowed in concentrations of 0.05 mg instead of the present 0.1 mg per m<sup>3</sup>. The levels of various solvents (acetone, toluene, formaldehyde) have also been reduced, as have octane-raising additives in fuel.

The new law has been criticised at the Health Centre of Stockholm's Institute of Technology, where it has been argued that the new levels are merely repetitions of those now in force in the United Kingdom and the United States and reflect more a balance between economy and hygiene than any facts about the substances cancer-producing properties. In spite of this, the measure will be welcomed by those for whom it will mean safer working conditions.

one of its type in the world. Gamma middle and high frequency levels.

rays from 179 radioactive cobalt (60Co) sources encased in a heavily shielded hemispherical central body are focused on to the tumour, which can then be PREVENTION may be better than cure, irradiated without significantly damagment. The latter may not themselves be dangerous, but may grow and put pressure on other nerves, affecting the patient's movement and senses. Victims patient will be fully conscious, and will radio.

> tion's Maritime Communications Satellite (MAROTS) programme which began in 1973. Although Norway only has observer status in ESRO, the country's large maritime fleet gives it a manifest interest in a civil maritime communications system. To seal its cooperation Norway will contribute 1.5% of the cost of the project, presently estimated to be of the order of \$80m. Sixty per cent of the total cost is being borne by the UK, with the balance being shared amongst the other participants: Belgium, France, Italy, the Netherlands, Spain, Sweden and West

> The MAROTS system will consist of ground facilities and one satellite in a geostationary orbit, expected to be positioned around 12.5°W. By means of a wide beam the satellite will cover most of the Atlantic Ocean, the western part of the Indian Ocean and the eastern part of the Caribbean region. The satellite, which will have a design life of not less than three years, will be put into orbit by a Delta launch vehicle from Kennedy Space Centre, it is hoped in mid-1977.

When it is operational, MAROTS will provide general communications, improved distress, search and rescue safety (including "all ships" information broadcasts and individual mobile weather routing by satellite) and evaluation of ranging techniques for line of position determination. The information the project will provide will help in the design of a future system, operating globally, intended to improve on MAROTS3 functions and to provide • Swedes who have contracted brain satellite capacity to relieve present contumours will after the New Year have gestion in the high frequency bands, the option of treatment by a new permit automation of radiotelephone fifteen-ton gamma-ray unit recently in- and teleprinter transmissions and cater stalled at the Karolinska Hospital. The for services such as high speed data design of the unit makes it the only transmission, not presently possible on

#### ARC faces money shortages squarely

by Eleanor Lawrence

THE practical effects of the Rothschild Report are now being felt by the research councils generally and by none more so than the Agricultural Research Council (ARC). Introducing the Annual Report for 1973-74, the Secretary of the ARC, Dr W. M. Henderson, was inevitably concerned with the effects of the resulting White Paper on the distribution of government funds for agricultural research, making themselves felt as they do at a time when there is less money all round.

Since 1967-68, as Dr Henderson points out, the annual growth rate of the Soience Budget from the Department of Education and Science for all the research councils had fallen from 11.2% to 3.9% and the ARC's share has decreased from 12.2% to 4%. The source of the funds available to the ARC is also changing. In 1973-74, 59% of its budget came from the Science Budget, 23% from the Ministry of Agriculture, Fisheries and Food (MAFF) and 18% from the Department of Agriculture and Fisheries for Scotland (DAFS). The expected proportions in 1974-75 are only 38% from the Science Budget, 46% from MAFF and 16% from DAFS.

The ARC's present programme of work has fitted in with the requirements of MAFF, the 'customer', without too much upheaval and new commissions from the ministry are scheduled to begin next April. Dr Henderson had special praise for the work of the Joint Consultative Organisation, a body made up of representatives of the ministries, universities, the research council and its institutes and units, and the farming community, which was set up to recommend priorities for future work and to evaluate the existing programme.

The tight financial position implies that no new work can be undertaken by the ARC. Although there seems to be no immediate danger of drastic cuts in particular projects, a reduction in the work force seems inevitable. At the moment there is a moratorium on the filling of posts left vacant through retirement or resignation, but Dr Henderson emphasises that this is not a satisfactory answer. "We cannot," he said, "under any circumstances stop the recruitment of a due proportion of good young scientists". He could, however, give no clear indication how this was to be achieved.

One of the most frustrating restrictions will be the inability to inject the necessary money at the 'growing' points where an increase in funding would bring in the best returns.

# correspondence

#### **UNESCO** and Trieste

SIR,—Your leader of November 8 was critical of the line you thought the United Kingdom would be taking on the question of the International Centre for Theoretical Physics, Trieste, at the recent 18th General Conference of UNESCO, and attributed this probable attitude largely to Sir Harold Thompson personally.

Now that the General Conference is over, we would like to put on record that Sir Harold Thompson, as the British spokesman in the Science Commission of the General Conference, was representing the views of the Royal Society UNESCO Committee, endorsed by Her Majesty's Government. Britain was not critical of the achievement of the ICTP: indeed the British delegate praised the Centre and its Director. Nor was the United Kingdom delegation seeking the withdrawal of UNESCO funds to the ICTP in the forthcoming biennium; it voted for the budget for the basic sciences, including the full proposed provision for the Trieste Centre in 1975 and 1976.

Along with several other Member States, however, the United Kingdom delegation at the same time supported a resolution, passed unanimously at the 94th Session of the UNESCO Executive Board in May of this year, which stated that:—

"(The Executive Board)... Takes the view that Unesco's continued support to the International Centre for Theoretical Physics, Trieste, for the years 1975-76, is acceptable, but is of the opinion that Unesco's relations with and subvention to the Centre should be reexamined carefully in the light of Unesco's total support to basic sciences."

D. C. MARTIN A. B. COZENS

Royal Society UNESCO Committee, Ministry of Overseas Development, London, UK

#### Irrationalism and science

SIR,—I agree wholeheartedly with the comments of Martin Raff (Nature, December 6). I have been to a number of good conjuring shows in my time and I have invariably found that in a high proportion of the tricks I am not only unable to see how they were done, but I have been unable to understand how they could possibly have been done without an entirely unreasonable amount of joint conspiracy with mem-

bers of the audience. In no case has this led me to believe that novel mental powers were required.

If we were to believe that Mr Geller's achievements went beyond our scientific understanding in the ways that his supporters suggest, there are some very uncomfortable corollaries which have apparently been unnoticed. For example, if he can bend a fork from a distance he could presumably be able to do the smaller mechanical task of putting a kink in an artery.

I am not imputing any undesirable behaviour to Uri Geller, but if other less upright exponents of his art should appear, could they be trusted not to try this on people who were in their way? We are better now at looking for reliable evidence for such things than were the past believers in black magic or present believers in Juju, and could investigate properly the statistics of unexpected coronary thromboses or strokes among the known enemies in the past of suspected people. Having found a number of suspects for which the probability of chance gave P < 0.001, we should still have around one in a thousand who would be unjustly indicated as guilty. For further tests the extensive mediaeval literature on witchcraft should be re-examined.

I seem to remember a technique which consisted of throwing the suspect into a pond; if she sank she was innocent and received a Christian burial while if she floated she was dispatched with a silver bullet and buried with a stake through her heart at cross roads.

Since I think the whole suggestion of super-normal powers is nonsense, I am not myself bothered, but those who take the opposite view might well be needing to consider just what legislation would be necessary for dealing with a witch if their case should ever be proved.

J. H. Fremlin

Department of Physics, University of Birmingham, UK

#### Strong, attractive, vain . . .

SIR, — The recent contribution by Gribbin¹ suggests, inter alia, that there may be a significance in the titular characteristics of the Leo sub-group on the Nature editorial staff. This possibility of significance is given weight by McIntosh, who writes of those born under Leo: "The Sun rulership gives him a strong attractive personality and makes it easy for him to command loyalty from others. These qualities

make him a natural leader, and the sign has long been associated with kings and potentates". It should perhaps also be mentioned that, according to the same source, "The faults that sometimes afflict the Leonian are vanity, pomposity and greed for power".

In view of the fit between sub-group attainment and expectations, it is regrettable that further research, carried out in an institution similar to the one in which Gribbin works, has shown that the Pisces hypothesis does not hold water.

MARTIN SHERWOOD

New Scientist, London, UK

 Gribbin, J., Nature, 252, 534 (1974).
 McIntosh, C., The Astrologers and their Creed, 130 (Hutchinson, London, 1969).

<sup>3</sup> New Scientist, 64, 880 (1974).

#### **Ghost authors**

SIR,—Ghost authors (*Nature*, December 6) are no new phenomenon. A good many years ago a paper by the late Professor Martin Rushton was published, giving after the author's name his qualification M.A.Cantab. Subsequently this was mistaken for a second author, and the reference "Rushton and Cantab" was a standing joke among dental research workers for some years. It found its way into at least one textbook.

DOROTHY A. LUNT
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#### Nullius in verba

SIR,—R. B. Cater's complaint (Nature. November 29) that 70.4% of all inquiries for a reprint spelt his name wrongly, is not as bad as be makes out.

It is a firm principle in science that the worth of any man's work is independent of that man's non-intellectual attributes, such as mere name. Far from displaying a lack of observational powers I would say that they are verifying the principle (and the motto of the Royal Society)—nullius in verba—on the words of no man.

Dr Cater would have had more cause for complaint if either (a) his work was disregarded because he was thought to be insignificant or (b) his work was accepted because his father was thought to have been a good scientist.

COLIN PRICE

The University, Hull, UK

# news and views

## Neuropharmacology of peptides

SEVERAL amino acids, such as glutamate, aspartate, glycine and gamma-aminobutyrate, have powerful excitatory or inhibitory effects on neuronal firing in the mammalian central nervous system. Recent findings have shown that certain peptides also have highly specific neuropharmacological actions, and it seems possible that some neurones may release peptides which have a neurotransmitter or modulatory role in the nervous system.

It has been known for some time that neurones in certain regions of the hypothalamus contain and release peptides with important endocrine functions. Thus neurones in the hypothalamus synthesise the hormones vasopressin and oxytocin which are released into the blood stream from the terminals of these cells in the posterior pituitary gland. Nicoll and Barker (Brain Res., 35, 501; 1971) found that vasopressin exerted potent inhibitory effects on the firing of neurones in the supraoptic nucleus, and suggested that vasopressin might be released by recurrent collateral fibres mediating inhibition of the neurosecretory cells. Vasopressin in low concentrations was also found to cause long lasting changes in pacemaker potential activity in a specific neurosecretory neurone of the land snail Otala lactea (Barker and Gainer, Science, 184, 1371; 1974). Other hypothalamic neurones secrete 'releasing factor' peptides into the portal circulation to the anterior pituitary and these peptides represent the final link whereby neural influences control the endocrine activity of this gland.

Recent findings suggest, however, that the neuropharmacological activity of certain hypothalamic releasing factor peptides may not be confined to the endocrine functions of the hypothalamic-pituitary system. The tripeptides TRH (thyrotropin releasing hormone) and MIF (melanocyte inhibitory hormone inhibitory factor) have a wide variety of behavioural effects in man and animals. In animals these peptides potentiate the psychomotor stimulant actions of Ldopa (Plotnikoff et al., Science, 178, 417; 1972) and enhance the behavioural excitation produced by a combination of tranylcypromine and L-tryptophan (Green and Grahame-Smith, Nature, 251, 524; 1974), and accelerate the rate of turnover of noradrenaline and dopamine in the brain (Friedman et al., Science, 182, 831; 1973; Keller et al., Nature, 248, 528; 1974). Several groups have claimed that TRH has transient mood elevating effects in depressed patients and in normal subjects (Prange et al., Lancet, i, 999; 1972; Tiwary et al., Lancet, ii, 1086; 1972; Wilson et al., Lancet, ii, 43; 1973; Wilson et al., Archs. gen. Psychiatr., 29, 15; 1973). The suggestion that TRH may have a role in controlling neuronal excitability in the central nervous system, apart from its endocrine function, is also supported by the recent report that more than two thirds of the total TRH in rat brain is located in brain regions outside the hypothalamus, with particularly high concentrations in certain septal and preoptic areas (Winokur and Utiger, Science, 185, 265; 1974; Brownstein et al., Science, 185, 267; 1974).

Another peptide with powerful neuropharmacological effects is the undecapeptide substance P, discovered by von Euler and Gaddum in 1931 in extracts of brain and small intestine (J. Physiol., Lond., 72, 74; 1931). The amino acid

sequence of substance P purified from bovine hypothalamus was reported by Chang et al. (Nature, 232, 85; 1971) and the synthetic material is now available. In this issue of Nature (page 734) Konishi and Otsuka show that substance P has a potent excitatory action on mammalian spinal cord motoneurones. They used an isolated spinal cord preparation from newborn rats, in which substances can be applied to the perfusion fluid in precisely controlled concentrations, and found that substance P was about 200 times more potent than the powerful excitant amino acid L-glutamate in causing a depolarisation of motoneurones. Substance P also facilitated monosynaptic spinal cord reflexes. In previous studies Konishi and Otsuka had shown (Brain Res., 65, 397; 1974) that substance P and the related peptides physalaemin and eledoisin were potent excitants of motoneurones in frog spinal cord, the latter compounds being 1,500-2,000 times more potent than L-glutamate. Takahashi et al. (Brain Research, 73, 59; 1974) also confirmed earlier reports that substance P is present normally in bovine dorsal root nerves, and that its concentration in these sensory nerves was 10-30 times higher than in the ventral root spinal motor nerve. These findings thus strongly support the view that substance P may be selectively concentrated in sensory nerves and that it could function as the transmitter substance released from sensory nerve terminals in the spinal cord, as suggested earlier by Lembeck (for review see Lembeck and Zetler, Int. Rev. Neurobiol., 4, 159; 1962). Substance P is also present in various regions of the brain, and in brain homogenates the peptide is highly localised in nerve terminal particles, or synaptosomes (Whittaker, Progr. Biopsys. molec. Biol., 15, 39; 1965). When applied iontophoretically from microelectrodes on to single neurones, in the cerebral cortex or cuneate nucleus, substance P has been found to be a powerful excitant of neuronal firing (Phillis and Limacher, Expl. Neurol., 43, 414; 1974; Krnjevic and Morris, Can. J. Physiol. Pharmac., 52. 736; 1974). The latter authors, however, found the actions of substance P to be slow in onset and longlasting, and suggest that the substance is unlikely to represent the quick acting transmitter released from sensory nerve terminals.

The possibility that 'peptidergic' neurones may exist, releasing peptide neurotransmitter substances, or that peptides may exert other long-term modulatory influences on neuronal function is one to delight the neuropharmacologist and the pharmaceutical industry. With modern techniques of peptide chemistry it should be possible to design and test a large number of structural analogues of the naturally occurring materials, and perhaps to develop neptides with highly selective neuropharmacological actions. For example, it may be possible to dissociate the behavioural and endocrine effects of substances such as TRH and MIF, or to obtain analogues of substance P that will penetrate more readily into the central nervous system from the circulation. If the initial promise is sustained, this seems likely to become an important new area for neuropharmacological research.

L. L. IVERSEN

#### Competition for laser fusion?

from a Correspondent

THE incident laser power needed to achieve a significant thermonuclear energy gain in a deuterium-tritium (DT) target which initially has a density near that of the solid state has been variously estimated to lie between 1013 and 3×1015 W; the lower power is appropriate to hollow spherical pellets seeded with materials having a nuclear charge greater than unity, and the higher power to pure, homogeneous spherical DT pellets. The predicted power needed to maximise thermonuclear gain from a given target depends not only on the sophistication and ingenuity of numerical or analytical models, but also on various physical assumptions; these may range from the technological, such as the inclusion of two-dimensional effects induced by small variations in incident light intensity or target symmetry, to the fundamental, such as a self-consistent treatment of non-linear laser-plasma phenomena. (For a good introduction to the subject, the reader is referred to review papers by J. Nuckolls, R. E. Kidder, K. A. Brueckner, R. L. Morse, and others, to be published in Laser Interaction and Related Plasma Phenomena, 3, edit. by H. Schwartz, Plenum Press.)

An interesting example of the interrelation between practical problems and more fundamental effects in laser fusion is afforded by recent radio-frequency and numerical experiments reported by Kim et al. (Phys. Rev. Lett., 33, 886; 1974), in which the trapping of radiofrequency electric fields within a density cavity, or 'caviton', has been demonstrated. Their experiment simulates the generation of a parametric instability which occurs when a non-uniform laserplasma is driven by a (pump) electric field directed parallel to the density gradient. Although this field component would be zero in an ideally symmetrical

spherical implosion, it may not be negligible in a practical experiment; the applied field is thus enhanced by linear, resonant conversion to electrostatic modes in regions near the critical density.

A wide range of other laser-plasma effects are also of interest. Consequently predictions of the laser energy needed for significant thermonuclear gain depend on the wavelength  $(\lambda)$  of the laser light. For example, Kidder estimated that, if core-corona decoupling is the limiting physical process in pure DT pellets, laser energy requirements range from 70 kJ (at  $\lambda = 0.265 \mu m$ ) to 3 MJ (at  $\lambda = 10.6 \mu m$ ), whilst Nuckolls computes that a 100 kJ, 1013 watt,  $\lambda = \frac{1}{2} \mu \dot{m}$  laser is required for useful power generation (invited paper given at VIII International Quantum Electronics Conference, San Francisco, June 1974). An economic DT fusion reactor thus requires lasers having energies significantly above those possible at present and with efficiencies of some 15% (Spalding, Energia Nucleare, 21, 176; 1974), although hybrid fusionfision energy schemes may lower the required laser efficiency (Horoshko, et al., Annals of Nuclear Science and Engineering, in the press). Probably for these reasons, Professor E. Teller recently told an audience at Stanford University that critical experiments in laser fusion may take a generation to complete (New Scientist, 64, 502; 1974).

Competition to lasers as a heat source for target compression in inertial confinement experiments comes from charged-particle beams, which can now be generated with very high electrical efficiencies. Considerable energies have already been delivered to small targets. Typical parameters for some present-day laser and relativistic electron-beam target-compression experiments are compared in Table 1. Assuming that ablation of a given target is self regulating, with energy deposition governed by the penetrating power of hot coronal electrons, the absorbed power requirement should be comparable for both laser and particlebeam approaches to inertial confinement. A fundamental problem for the particle approach may thus prove to be the generation of sufficiently high power beams, and their subsequent application at high, uniform intensities to sub-millimetre targets. Winterberg has recently made the interesting suggestion that 1-10 MV heavy ions, having an energy distribution which is instantaneously mono-energetic but which varies with time, might provide a convenient means for axial (time) compression of the beam after passage through a neutralising drift tube (Nature, 251, 44; 1974). Although 300 keV proton beams of approximately 75 J energy have recently been produced with an efficiency of 40% (Bull. Am. phys. Soc., Series II, 19, 871; 1974; papers 2C 14 and 15) no experimental attempt at pulse compression of heavy ion beams has yet been reported. If suitable high-intensity ion-beam techniques could be developed and successfully demonstrated in a compression experiment, the relatively high efficiency with which power could be deposited in the target might provide strong competition to other inertial-confinement approaches.

esent laser tech-In summary niques seem to offer the most immediate prospect for generating very high density plasmas. Significant laser developments will however be required to permit high-efficiency heating of isolated fuel targets, and to face a potential challenge from charged particle beam techniques for practical fusion energy applications. The likely time scale and scientific challenge to both approaches closely parallels current controlled thermonuclear reactor magnetic confinement programmes.

#### Probing nuclear band structure

from P. E. Hodgson

Many nuclei have low-lying states that are readily interpreted as members of a rotational band. Their energies follow quite closely the values expected from the quantum-mechanical theory of a rigid rotator, and their spins follow typical rotational sequences like  $0^+, 2^+, 4^+ \dots$  or  $\frac{1}{2}^+, 3/2^+, 5/2^+ \dots$  depending on the spin of the lowest member of the band. Furthermore, the transition probabilities for the excitation and de-excitation of these bands are found to be in good accord with the same quantum-mechanical theory.

Many such bands have now been found, especially in the regions of the periodic table far from the closed shells where nuclei are frequently deformed. But though it may be easy to identify the lower members of a band it is

Table 1 Some typical target compression experiments

Tabi	o x Some typ	near target comp	ression experime	1113	
Beam characteristics	Number of beams	Total energy (J)	Incident power (W)	Incident intensity (W cm -2)	Ref.§
1* λ = 1.06 μm $2* λ = 1.06 μm$	2	230 1,000–3,000	$2.0\times10^{11}$	$4.0 \times 10^{15}$	F1 F3.2
$3* \lambda = 1.06  \mu m$	4	800	$2.0 \times 10^{12}$	$2.0 \times 10^{16}$	F4.2
$4 \lambda = 0.53 \mu m$	4	140		_	F4.2
5* λ == 1.06 μm	12	1,400	$4.0 \times 10^{11}$		F4.3
$6* \lambda = 1.06  \mu m$	4	320	$1.5 \times 10^{11}$	1015	F4.4
$7* \lambda = 1.06  \mu m$	2	50	$1.7 \times 10^{12}$	• –	F4.5
8† $\lambda = 10.6  \mu m$	1	200	$1.4 \times 10^{11}$	$7.0 \times 10^{14}$	F4.5
9‡ 4 MV electrons	1	1,000	$\sim 5.0 \times 10^{10}$	$\sim 10^{12}$	F2.1
10‡ Î MV electrons	2	20,000	< 1012	_	F2.2

Nd-glass laser efficiency  $< \frac{1}{2}\%$  CO<sub>2</sub> laser efficiency currently of order 2% (can be improved)

Electron beam efficiency of order 50%

Paper number from the Fifth Conference on Plasma Physics and Controlled Nuclear Fusion Research, IAEA-CN-33, 11-15 November 1974, Tokyo

often difficult to identify the higher members as there may be several states of the appropriate spin with approximately the right excitation energy. The identification is frequently made more difficult by quantum-mechanical mixing of the states of the same spin belonging to different bands that perturbs the simple sequence of energies.

Some recent work by Anantaraman and colleagues at the University of Rochester (Phys. Rev. Lett. 33, 846; 1974) has shown that the (6Li, d) reaction can sometimes be used to assist the identification of members of a particular band. Previous work had already shown that this reaction is sensitive to the transferred orbital angular momentum, indicating its usefulness in nuclear spectroscopy. This means that when a nucleus is bombarded with 6Li ions many deuterons are emitted and the angular distributions of the deuterons of a particular energy leaving the residual nucleus in a particular state is characteristic of the orbital angular momentum L given in the reaction to the target nucleus. If the target has zero spin, the orbital angular momentum couples vectorially to the spin of the transferred particle giving the spin of the final state  $J=L\pm\frac{1}{2}$ . This ambiguity can usually be resolved by shell model considerations, or by other measurements, such as that of the polarisation of the emitted particle. If the spin of the target nucleus is not zero several orbital angular momenta can contribute and they can easily be separated if they have similar magnitudes and different shapes, as is often the case. As before this determines or sets limits to the spin of the final state.

The Rochester group studied the reaction  $^{29}\text{Mg}(^{9}\text{Li}, d)$  to states of  $^{29}\text{Si}$ . It was already known that the rotational model is able to describe some of the low-lying states of  $^{29}\text{Si}$  by assuming that they belong to oblate  $K=\frac{1}{2}^{+}$  and  $3/2^{+}$  rotational bands. The  $K=\frac{1}{2}^{+}$  band is based on the ground state and has members  $\frac{1}{2}^{+}$  (0.0 MeV),  $3/2^{+}$  (2.43

## Glow discharge optical spectroscopy

from John Walker

Transistors, integrated circuits, solid state lasers and many other electronic devices are made by introducing impurities into silicon, germanium and compound semiconductors like gallium arsenide. The impurities are usually diffused in at a high temperature or are ionimplanted (fired into the material at a high speed after being accelerated by an electric field). In either case the impurity atoms are not distributed uniformly throughout the semiconductor crystal—their con-centration varies with depth. The precise variation (the 'profile') needs to be known, because it affects the production yield and the quality of the device. In general, profiles cannot be calculated accurately: they must be measured. Although various techniques are available, in one technologically important case. boron in silicon, they are either not applicable, too inaccurate, or complicated and expensive.

To resolve this problem a group of workers at the University of Illinois (Greene et al., Applied Physics Letters, 25, 435; 1974) have devised a modification of the wellknown technique of spectrographic analysis which they call "glow disoptical spectroscopy" charge (GDOS). The boron-doped silicon sample is bombarded by argon ions in an electrical discharge. This knocks atoms out of the sample, which are then excited in the discharge and emit light of particular wavelengths. Just as sodium street lamps give out an orange light characteristic of sodium atoms, so a boron discharge emits ultraviolet light at a particular wavelength. The more boron atoms there are in the silicon crystal the more intense the light emitted. Furthermore the crystal surface is gradually worn away by the boron ion bombardment, so that the change in boron concentration with depth can also be investigated.

When the method was applied to a diffused sample the measured boron profile was found to depart from the theoretical distribution by a factor of ten at some depths, confirming the inadequacy of calculations. A second silicon wafer, into which boron had been ion-implanted, had a maximum concentration at a depth of 430 nm, in agreement with calculations, but some boron had penetrated much further into the crystal than the simple theory predicted—an observation also made by other workers.

These results indicate the effectiveness of GDOS in detecting and profiling boron impurity in silicon. The method can be used on other impurities, and on compounds. For example, it has been applied to tin in gallium arsenide. Insulators can also be tested, and impurities need not be electrically active. The equipment is relatively simple, and available in most laboratories. Glow discharge optical spectroscopy obviously has a bright future, particularly in the electronics industry.

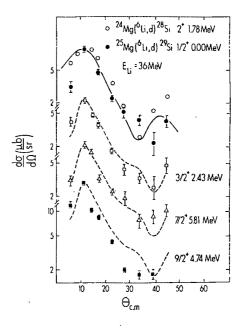


Fig. 1 Differential cross sections for the <sup>24</sup>Mg(<sup>8</sup>Li, d)<sup>28</sup>Si reaction to the 2<sup>4</sup> state at 1.78 MeV and for the <sup>23</sup>Mg(<sup>8</sup>Li, d) reaction to the states of <sup>29</sup>Si forming the K=½<sup>+</sup> band. The full curve is a distorted wave calculation of the cross section of the first reaction, and the dashed line is a smooth curve drawn through the data for the second reaction to the ½<sup>+</sup> state. All these reactions thus have the angular distribution characteristic of L=2 transfer.

MeV),  $5/2^+$  (2.03 MeV) and  $9/2^+$  (4.74 MeV), and the K=3/2 band has members  $3/2^+$  (1.27 MeV),  $5/2^+$  (3.07 MeV).  $7/2^+$  (4.08 MeV),  $9/2^+$  (5.65 MeV) and  $11/2^+$  (7.14 MeV). The energies are not in regular sequences due to the considerable band mixing. There is a missing  $7/2^+$  member of the K= $\frac{1}{2}^+$  band and there are several possible candidates, but it was not known which is the member of the band and which belong to quite different configurations.

Studies of the angular distributions of the deuterons from the reactions that go to each state in the  $K=\frac{1}{7}$  band showed that they are all the same and have the characteristic shape of an L=2 transfer. This shape is known from studies of the 24Mg(6Li, d)28Si reaction to the 2+ state at 1.78 MeV, which can only go by L=2 transfer. The 25Mg(6Li, d) reaction to the 1+ ground state of 29Si can also go only by L=2 transfer, because the spin of <sup>25</sup>Mg is 5/2+, but all the other reactions to higher members of the  $K=\frac{1}{2}$  ground state band can in principle go by several different values of L. Thus for example the reaction to the 3/2+ state can go by L=2 or 4, as these are possible solutions of the vector equation 5/2+L= 3/2+ (the odd values of L are excluded by the requirement of parity conservation). Nevertheless as shown in Fig. 1 only the lowest L=2 transfer is found in this and in all the other transitions

to this band. Among the possible candidates for the  $7/2^+$  member of the band is the state at 5.81 MeV that shows just the same angular distribution; this is suggestive but not conclusive as not all the  $7/2^+$  states were resolved in the experiment, so other candidates are possible.

In sharp contrast to this uniform L=2 behaviour the angular distributions of the <sup>23</sup>Mg(<sup>6</sup>Li, d) reaction to states of the K=3/2<sup>+</sup> band, shown in Fig. 2, have the irregular behaviour characteristic of a mixture of the allowed L-values. The curves in the figure correspond to various mixtures of .L-values, as determined by the <sup>24</sup>Mg(<sup>6</sup>Li, d) reaction. This sharp difference in the behaviour of the reactions to these two bands is all the more remarkable in view of the strong mixing between the two bands.

The mixing of the L-values for the transitions to the  $K=3/2^+$  band shows that the predominance of L=2 in the transitions to the  $K=\frac{1}{2}$  band is not due to kinematics but must therefore be due to differences in nuclear structure. At present there is no explanation for this behaviour, and it shows the need for more detailed nuclear structure studies.

It is, however, not necessary to understand the reason for the different behaviour of the transitions to the two bands in order to use it empirically as a way of identifying the different bands. It is likely that many other bands will be found to have characteristic signatures, and that these reactions will provide a useful way of studying the structure of rotational nuclei. It will also stimulate nuclear reaction calculations to try to understand the difference in the structures of the bands that give rise to this effect.

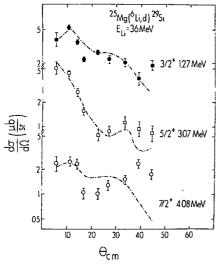


Fig. 2 Differential cross sections for the <sup>25</sup>Mg(<sup>6</sup>Li, d) reaction to the states of <sup>29</sup>Si forming the K=3/2+ band. The curves for the 3/2+ and 7/2+ states are mixtures of L=2 and L=4 distributions and that for the 5/2+ state is a mixture of L=0 and L=2 distributions.

# Structure of molecules in solution

from J. Feeney

To reach an understanding of biological recognition processes such as those involved in enzyme-substrate hormone-receptor interactions will require a detailed knowledge of the three-dimensional structures and the potential flexibility of the component molecules. Inspired by this challenge, Dr R. E. Richards, FRS and Professor R. J. P. Williams, FRS organised a meeting on November 21 at The Royal Society to discuss the present status of the various experimental methods for determining structures and conformations in solution. Although it is clear that none of the available methods offer the high precision of structural information provided by X-ray diffraction, some of these methods allow one to answer the important question of whether the unique conformation present in the crystal is the only one present in solution. Several speakers explored the applications of nuclear magnetic resonance (NMR) spectroscopy to these problems and this method emerged from the discussion as the dominant technique for providing conformational, dynamic and ionisation information for molecules in solution.

R. J. P. Williams (University of Oxford) illustrated how the conformation of ligands such as nucleic acids and peptides can be obtained from the chemical shift and relaxation time perturbations caused by the binding of a paramagnetic lanthanide ion to the molecule. When the interactions can be shown to be dipolar in origin then the chemical shift perturbations depend on  $r^{-3}$  where r is the internuclear distance between the lanthanide ion and the perturbed magnetic nucleus (proton, carbon, phosphorus) and an orientational term  $(3\cos^2\Theta - 1)$  where  $\Theta$  is the angle between this internuclear vector and the principal symmetry axis of the complex. Relaxation changes when using a lanthanide ion such as Gd3+ are isotropic and depend on  $r^{-6}$ 

A computer-simulated NMR spectrum is then generated for the fixed conformation which best fits the observed data. Only if there is an excellent fit for both the chemical shift and relaxation time perturbations can one assume that there is predominantly a single conformation in solution. • It became clear from the discussion that it is possible by careful experimentation to demonstrate the validity of the approach for rigid molecules. But for potentially flexible molecules such as peptides, the 'conformation' as formed by this technique is not simply related to the distribution of conformations but probably reflects the most populated conformation.

J. Feeney (National Institute for Medical Research, London) described an alternative NMR method of obtaining conformational information involving the well-established procedure of relating measured three-bond H-H spin-coupling constants ( $^{3}J_{HH}$ ) to the dihedral angle \varphi about the central bond. When the form of the  ${}^3J_{\rm HH}/\varphi$ relationship (the so called Karplus equation) is known for a particular system it can be used to define dihedral angles in molecules of fixed conformation. For flexible molecules it is possible to estimate the fractional populations from the averaged coupling constants and this allows one to check potential energy calculations. The method has been extended to three bond coupling constants involving other nuclei (1H-13C, 1H-31P and 1H-14N). Various applications to cholinergic neurotransmitters and hormonal peptides were considered. It is clear that for small molecules which are potentially flexible a combination of the spin-coupling constant approach with the lanthanide induced shift measurements is required to obtain the maximum amount of conformation information.

For larger molecules such as the enzyme lysozyme where one expects a unique conformation in solution, the full potential of the lanthanide induced shift technique can be realised. C. M. Dobson and I. D. Campbell (University of Oxford) described how the spectral perturbations caused by binding lanthanide ions to hen egg white lysozyme in solution could be interpreted in terms of the crystal structure of lysozyme, thus indicating that the solution and solid state conformation are very similar. By the elegant use of NMR difference spectroscopy most of the proton resonances in close proximity to the lanthanide ion binding site can be assigned with confidence. Dr Cambell showed how many other proton assignments in lysozyme can be made independently of the X-ray diffraction crystal structure. By using high magnetic fields and computational methods of resolution enhancement many of the proton resonances could be resolved and assigned from a consideration of their multiplet structures, and results from double resonance methods, pH titrations, chemical modifications, paramagnetic probes and inhibitor binding. On the basis of these experiments it is clear that this method can be extended to proteins where the X-ray structure is not yet available. As pointed out by Campbell and Dobson, the assigned resonances may be used to monitor interactions between catalytically important groups, to determine their ionisation states and to deduce the mobilities of the side chains of certain residues.

T. G. Spiro (Princeton University) showed how resonance Raman spectroscopy of haem proteins can be used to detect deviations from planarity of the porphoryin ring and other structural consequences of changing the spin and oxidation states in the haem groups. The vibrational modes containing this information exhibit greatly enhanced Raman scattering when an electronic transition to which they are coupled is irradiated with laser excitation.

X-ray and neutron scattering techniques were considered by J. E. Enderby (University of Leicester) and J. W. White (University of Oxford). Because the neutron diffraction scattering length for the proton is opposite in sign to that for deuterium, by using mixtures of H<sub>2</sub>O/D<sub>2</sub>O it is possible to achieve high contrast between the scattering from the solvent and from the solute molecules. For inelastic scatter-

ing there is a large difference in cross section between protons and deuterons which allows observation of the dynamic behaviour of individual components in the various phases of amphiphile/water systems. Low angle neutron diffraction studies on hydrated collagen and tobacco mosaic virus solutions lead to information about the radii of gyration of the molecules in solution.

From measurements of the quasielastic spectrum of laser light scattered from macromolecules in solution, G. B. Benedek (Massachusetts Institute of Technology) showed how accurate diffusion coefficients can be obtained. When considered together with sedimentation data these values provide molecular weights. The size and shape of the macromolecules can also be deduced. Interesting differences in the apparent volumes of lysozyme when denatured by different methods (guanidine and heat) were observed but no evidence for intermediate states in the unfolding process could be detected. The technique is capable of monitoring aggregation of macromolecules and this was well illustrated by a study of the manner in which the aggregation of glutamate dehydrogenase controls the amount of enzyme in its active form.

Most of the techniques considered have still to reach their full potential in their application to structure determination in solution and rapid progress can be anticipated over the next few years.

# Huntingtons's chorea and GABA

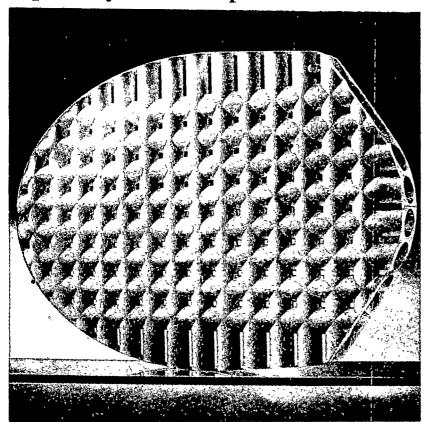
from T. J. Crow

THE apparent diversity of chemical neurotransmitter substances (there are now at least seven with good credentials) in the brain has encouraged the search for specific neurohumoural disunderlying neurological turbances diseases of obscure actiology. Ehringer and Hornykiewicz' discovery (Klin. Wschr., 38, 1236-1239; 1960) of a deficiency of dopamine in the basal ganglia in Parkinson's disease led to the introduction of L-DOPA in treatment. Now it is suggested that another progressive disease of the 'extrapyramidal' motor system, Huntington's chorea, is associated with a specific deficiency of GABA (γ-amino-butyric acid) in the same brain regions.

GABA, known for 20 years to be present in brain, has a regional distribution which follows that of its synthetic enzyme glutamine acid decarboxylase (GAD), and both are present in synaptosomal fragments. GABA is in high concentration within the striatum, globus pallidus and substantia nigra, structures within the extrapyramidal motor system, and also within the cerebellar cortex and nuclei (Fahn and Coté, J. Neurochem., 15, 209-213; 1968). The presence of a high-affinity uptake system and the demonstration of release following neural stimulation provide further evidence that this substance has a neurotransmitter role, and microionophoretic data suggest that at several sites GABA is an inhibitory transmitter. The recent development of an immunohistochemical technique by Saito et al. (Proc. natn. Acad. Sci. U.S.A., 71, 269-273; 1974) for GAD may greatly facilitate the task of localising particular gabaminergic systems.

Huntington's chorea is transmitted as an autosomal dominant condition which may affect between 4 and 7 per 100,000 of the population. The disease owes its persistence, and its peculiarly sinister character, to the fact that the average age of onset (44 years, according to Wendt) is beyond the normal period of fertility. Thus by the time symptoms appear the patient may have

## Optical systems for space



• photograph by Tinsley

The mirror illustrated (made by Tinsley in Berkeley, California) is designed to be part of a rocket-launched optical system. The grid pattern is formed by a series of holes drilled through the core to minimise weight. Because of the high stresses experienced in a large object during a launch, the accuracy of machining is critical and is evident in the regularity of intersection of the holes.

children who in turn face the prospect that they may develop the condition. The disease has both neurological and psychiatric aspects in that the development of jerky, uncontrolled, choreiform movements precedes a steady progression into dementia. Panse (Die Erbchorea; Leipzig, 1942) has demonstrated that a substantial minority of cases pass through a paranoid psychotic phase with schizophrenia-like features early in the illness. An understanding of the mechanism of these changes might have implications beyond the field of motor control.

Perry et al. first reported a deficiency of GABA in the brains of patients dying from Huntington's chorea in 1973 (New England J. Med., 288, 337-342). Later that year Bird and his colleagues (Lancet, i, 1090-1092) and McGeer et al. (Neurology, 23, 912-917) reported a reduction in the levels of GAD, which is presumed to be a marker for gabaminergic neurones. Now Bird and Iversen (Brain, 97, 457-472; 1974) have presented a detailed study of the brains of 38 patients dying with the disease and 38 controls of similar age dying from other causes. In this study the time between death, postmortem and subsequent freezing of the tissue was kept to a minimum. Glutamic acid decarboxylase activity in the choreic patients was reduced by 75% in the basal ganglia, but not in the frontal cortex. GABA concentrations were reduced by 50%, and choline acetyltransferase concentrations by 55%, although these latter were within the normal range in a substantial proportion of the brains. Tyrosine hydroxylase activities and dopamine concentrations did not distinguish the two groups although a subgroup with rigidity (a possible variant of the disease) had significantly higher dopamine concentrations. Bird and Iversen argue that the most consistent lesion in Huntington's chorea may be a loss of GABAcontaining neurones, with, in some cases, a loss of cholinergic neurones. In a study on part of the same postmortem material Hiley and Bird (Brain Res., 80, 355-358) have shown that there is sometimes a loss also of the cholinergic muscarinic receptor. That this post-synaptic change may precede the loss of the pre-synaptic cholinergic neurone is suggested by the observation that normal choline acetylase levels are sometimes present with reduced concentrations of the muscarinic receptor.

These findings provoke a number of questions concerning possible neuro-chemical-clinical correlations. For example although there is electrophysiological evidence for a role for GABA-containing neurones in the cerebral cortex, there is no evidence in the recent study that these neurones are affected by the disease process. Therefore on

the evidence thus far available the dementia of the disease must be explained on the basis of subcortical changes, or some non-gabaminergic cortical cellular dysfunction. Second, there has been considerable interest in GABA in the cerebellum (a paper by Wilkin and co-workers in a recent issue of Nature (252, 397; 1974) presents convincing radioautographic evidence that the inhibitory Golgi cell terminals on the granule cell dendrites are one cerebellar site at which a high affinity GABA-uptake system is present). Huntington's chorea is not generally considered to be a cerebellar disease, but there is sometimes histopathological evidence of cell loss in this structure. Is it possible that such cell loss, when it occurs, is also of GABA-containing cells? Finally one might speculate concerning the paranoid psychotic changes which are occasionally seen early in the disease. Recently there has been interest in the hypothesis that excess dopaminergic activity underlies the paranoid form of schizophrenia. As Bird and Iversen point out the histopathological atrophy in Huntington's Chorea may lead to a relative increase in the density of innervation by surviving nigro-striatal dopaminergic terminals. Therefore a neurohumoural imbalance at this level provides a possible basis for the development of a paranoid psychosis which in turn may disappear when continued cell loss leads to the supervention of dementia.

# Superconducting flux structure probed with positive muons

from P. G. Harper

ONE of the characters making a brief appearance in the drama of parity nonconservation (1956) was the positive muon  $(\mu^+)$ . This fundamental particle is analogous to the positron, but differs in mass (about 130 times larger), and attendant decay neutrinos. It is emitted from the decaying positive pion  $(\pi^+)$ in a spin-polarised state, that is to say, with its magnetic moment tending to lie along its direction of propagation. The  $\mu^+$  is relatively long-lived with a half-life of 2.2  $\mu$ s, and decays by positron (e+) emission. The angular distribution of the latter is anisotropic with respect to the direction of  $\mu^+$  polarisa-

These basic properties of the  $\mu t$  enable it to be used as a probe to examine magnetic materials such as Fe and Ni. and very recently (*Phys. Rev. Lett.*, 33, 969; 1974), the type II superconductive flux structure. The principle is that if a  $\mu^+$  rests in a local magnetic field, its magnetic moment (or spin axis) will rotate (precess) about

the field direction at a rate proportional to the field. Because of the emission anisotropy, the instantaneous axial tilt at the moment of decay imparts a corresponding perturbation to the e<sup>+</sup> angular distribution. The positron emission, detected by coincidence counters in some fixed direction, thus follows the  $\mu^+$  precession so that the overall time decay is modulated at the precession period. For a field of 500 G, this would be about 0.01  $\mu$ s. Since the gyromagnetic ratio for  $\mu^+$  is known with considerable accuracy, the local magnetic field can be readily deduced.

The method thus resembles the socalled 'time-dependent perturbed angular correlation' technique employed with radioactive species. The advantage of a  $\mu^+$  beam is that it does no damage and leaves no residual contamination. Of course, when the method is employed to probe a non-uniform field distribution, the e<sup>+</sup> count records a space-averaged precession so that the frequency spectrum of the modulation requires careful interpretation.

The superconductive flux structure mentioned above is perhaps the most bizarre magnetic ordering found in any low temperature solid state phase. Present physical understanding of this phenomenon is fairly complete, and is hased on developments of the remarkable 1950 theory of Ginsberg and Landau. Whereas for a thick specimen of lead, say, in its superconductive state, a weak applied magnetic field may be completely internally extinguished within 0.1  $\mu$ m of a parallel surface, in a type II material such as a lead-indium alloy, a similiar field can penetrate right across the interior. It succeeds however only by condensing regularly into quantised filamentary flux lines (or vortices) which run parallel to the external field; they form a two-dimensional hexagonal structure with a flux-line density equal to the ratio of total flux to the universal flux unit, hc/e. At the centre of each flux line, the material is actually in its normal state, so that the induction achieves its maximum (that is, external) value. Away from the centre the induction declines, while at the same time the degree of superconductivity increases. Near the mid-point of neighbouring centres the induction forms a saddle-point. A strong hope for the  $\mu^+$ technique is that it might reveal and measure the saddle-point local field.

Of course, other methods have been used to probe the flux distribution, notably NMR, neutron diffraction, molecular beams and decoration microscopy. The present experiment therefore is largely to test a fairly novel technique, though additionally, it reveals some features of  $\mu^+$  behaviour.

The alloy  $Pb_{0.90}I_{0.01}$ , and Nb were.

examined for comparison in the normal state, with the field applied, and with same field in the superconductive state. The latter was prepared by (1) cooling in the steady field to the mixed state, and (2) cooling in zero field, and then switching on. The results for the alloy were encouraging. For procedure (1), the main Fourier component of the modulated count (corresponding to the frequency of the applied field) was observed to broaden upon cooling to 5.5 K: upon further cooling to 3 K, a bifurcation was resolved, and a satellite peak identified as arising from the saddle-point mentioned above. Procedure (2) however showed no such splitting, indicating that the structure was smeared out by the preparation. Less exciting results were found for the Nb. No splitting was observed with either (1) or (2), but the main (external field) line appeared with much larger amplitude.

The authors conclude their report with a discussion concerning the possible diffusion of the  $\mu^+$ . Far from being simply trapped at a favourable location the tunnelling rate between Nb sites is estimated as being at least  $10^{12}$  s<sup>-1</sup>. If the observed sharp line in Nb is attributed to motional narrowing (as occurs in NMR for example) a large diffusion constant of the order  $10^{-2}$  cm<sup>2</sup> s<sup>-1</sup> is needed. At present, actual diffusion mechanisms remain speculative and the authors plan further work to resolve them.

#### **Comet chemistry**

from M. K. Wallis

"THE Study of Comets", The International Astronomical Union Colloquium No. 25, held from October 28 to November 1 at NASA's Goddard Space Flight Center in Maryland, was timed to take stock of new data from comet Kohoutek and also to precede critical decisions on a possible cometary space probe.

Most of the active comet researchers from the United States and Western Europe put in an appearance, but unfortunately, in spite of sponsorship from the IAU and the International Committee on Space Research, nobody from the strong Soviet and Czech groups attended.

Much interest was attracted by the reports of the new molecular emissions, revealed in the ultraviolet and radio spectra. The 1,304 and 1,657 Å multiplets have given data on atomic oxygen and carbon, reported P. D. Feldman of Johns Hopkins University. And the radio observations, reviewed by L. E. Snyder (University of Virginia) have revealed CH, OH, HCN and CH<sub>3</sub>CN in comet Kohoutek and H<sub>2</sub>O in comet

Bradfield. Quantitative interpretation is thwarted however, as all except the HCN emission is pumped. The repeated failure to detect the predicted formal-dehyde (H<sub>2</sub>CO) emission is also put down to pumping.

Surprisingly, there is also a new identification in the optical—of H<sub>2</sub>O<sup>+</sup>—a joint effort with P. Benvenuti (Asiago Observatory, Italy) and P. Wehninger (University of Tel-Aviv) as G. Herbig of Lick Observatory related. There are numerous lines from this ion, from the head and from far in the tail. They can be found on old spectra and the ion is probably very common as cometary plasma tails frequently show up on red-sensitive photographic plates, which should exclude the normal CO<sup>+</sup> emissions.

Arpigny of Liège (Institut C. d'Astrophysique) reported progress in the interpretation of molecular spectra. in particular in modelling collisional changes to the relative populations and line strengths of the purely resonant fluorescent description. Applying this potentially powerful method to the CH bands has given a reasonable total density in comet Bennett, with temperature of the colliding agent in the range 4-800°K. Analyses of the extensive H-coma were reported by H. U. Keller of LASP (University of Colorado), who has made this topic his own. All observations give outflow velocity in the range 7-10 km, which may correspond to the as yet unknown surplus energy of OH photodissociation. But the distant Lyman-a profiles of comet Bennett, observed from OGO-5, require for their explanation an atomic H component of higher velocity, around 20 km s<sup>-1</sup>, as would be expected from the photodissociation of H<sub>2</sub>O.

Including the new identifications. A. H. Delsemme (University of Toledo) advocated the simple two-stage picture of the cometary coma: H<sub>2</sub>O and certain unknown parent molecules expand radially outwards and dissociate into daughter radicals and atoms, which are subsequently destroyed by ionisation. He gave length scales for the hypothetical parents and observed daughters. But a paper by C. K. Kumar and R. J. Southall of Howard University reported widely different scales in various comets for the supposed parents of CN and C. And a chemist, R. Oppenheimer of Harvard, emphasised in a spirited contribution that numerous ion-molecule and dissociation-recombination processes can proceed rapidly inside 10.000 km in the coma. Various complex reaction chains could occur, resulting in CH2 CN. C2 and, in principle, even H<sub>2</sub>O! The fundamental constituents of comets are still very much in doubt.

H. U. Schmidt of Munich (Max-Planck Institut für Physik und

Astrophysik) reviewed progress in the fluid description of plasma motion through the coma. He considered that magnetic stress from the distorted field of the solar wind would reduce the distance of the contact discontinuity dividing off the purely cometary plasma, but explanations of how structures were produced from this plasma and gave rise to the observed tail were still lacking. M. K. Wallis (University of Oxford) argued, however, that because of cooling and recombination processes, the 'contact discontinuity' would occur inside the 10,000 km collisional region and therefore have little significance.

Sequences of pictures of ion tails of various comets had been made into a film by K. Jockers (Sacramento Peak Observatory). This illustrated particularly that subsidiary systems of rays sometimes arise from condensations in the tail and that nearby condensations and wave features move at similar speeds, so would appear to be convected features in a general flow. Progress was being made in the theory of dust tails, reported Z. Sekanina (Smithsonian Astrophysical Observatory). The fact that the 'silicate signature' in the infrared emission is not present in the anti-tail proves the particles differ in size.

There was as ever speculation about processes of comet formation: A. Mendis (University of California, San Diego) suggested that they could be accreted on the time scale of 10<sup>3</sup> years in present-day meteor streams; F. L. Whipple (Smithsonian Astrophysical Observatory) wished to use the compression of a solar gale from the young sun, while B. Donn (NASA: Goddard Space Flight Center) reckoned comets might be formed in a dense cloud within the cluster from which the sun emerged.

As to the long discussed mission to a comet, a space probe to comet Encke in 1980 now has a reasonable chance, reported I. Rasool (NASA). With a 'slow fly-by at 8 km s<sup>-1</sup>, the nucleus could be photographed and the neutral coma and plasma coma and tail could be investigated.

It is aimed to publish both impromptu and scheduled talks, by courtesy of NASA. in what will surely be voluminous conference proceedings.

#### Correction

The source of the illustrations from the ceiling of the British Museum (Natural History) (Nature, 252, 193; 1974) was not acknowledged: they were reproduced from the Botany Leaflet No. 1 published by the British Museum (Natural History).

# articles

# Pupils of a talking parrot

Richard Gregory\* & Prue Hopkins†

Chance observations on a talking parrot have led to an investigation which reveals a striking temporal relationship between pupillary constriction and learned utterances.

A FEMALE talking parrot of the species known in this country as Yellow Fronted Amazon, Panama variety (Amazona ochrocephala panamensis), about nine years old and owned for the last five by one of us, exhibits marked constriction of the pupils of the eyes when she talks. She has a repertoire of about twenty words, calls, whistles and various recognisable sounds which are frequently uttered—accompanied by sudden and large pupil contraction. Once noticed, this is unmistakable, at least in Seraphita.

Although normally the pupils remain of almost constant size, even over large changes of illumination, during utterances

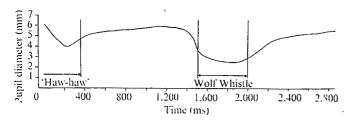


Fig. 1 Sample of analysed record (smoothed) showing shrinking pupil immediately preceding and during parrot's utterances.

they shrink rapidly to about half their resting diameter. This was such a clear and repeatable phenomenon that we decided to record it, and especially to discover time relations between the pupil shrinking and speech. During these investigations we found that the pupils would sometimes respond similarly while the parrot was listening to familiar words or sounds. Further, occasionally there would be a small shrinking of the pupils immediately prior to her speaking; as though, we thought, following some kind of internal 'rehearsal' for speech production. These further effects are smaller and more variable than the original effect observed, but after many hours of observation we are confident of them also, and have film records of their occurrence. It is known that the iris muscles are striated1, which helps to explain the lability. There is no consensual reflex: varying illumination to one eye does not produce pupillary changes in the other eye. With the speech changes, however, both pupils move together.

The effects were recorded on several occasions, over a period of two years, on video tape with sound track. Technically satisfactory sections of the video tape records were transferred to 16-mm film, with magnetic sound strip. Transfer to film was undertaken because freezing the video tape on the video tape recorder (1-inch Ampex) gave an unsteady picture and \*MRC Brain and Perception Laboratory, Department of Anatomy, The Medical School, University, Walk, Bristol BS8 1TD. †(Seraphita's home) White Gates, 4 Church Lane, Abington, Cambridgeshire.

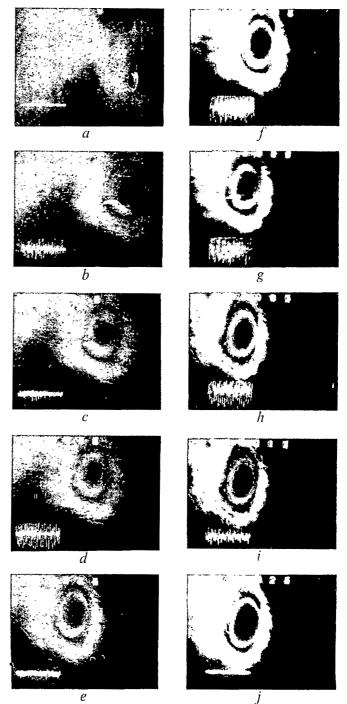


Fig. 2 Sequence from cine film of video tape, with oscilloscope record of parrot's 'wa-ha-he-e-y', electronically mixed in real time a, Time 0; b, 240 ms; c, 400 ms; d, 480 ms; e, 720 ms; f, 1,000 ms; g, 1,240 ms; h, 1,520 ms; i, 1,760 ms; j, 2,080 ms.

Speech started at 160 ms.

caused tape damage. The transfer allowed us to superimpose an oscilloscope record of the sound track, for measuring time relations between speech and pupil changes. The film camera was synchronised at 25 frames s<sup>-1</sup>, to the 25 s<sup>-1</sup> frame scan of the video system allowing time intervals to be estimated in 40 ms steps. The stop-motion projector has no sound head, so each section of speech was first classified, on an editor with a magnetic sound head (whose viewing screen was however too small for analysis) and a tape cassette of the sound track was also made for later checks. The moving inner edge of the iris—the pupil—and the unchanging outer edge of the iris of a single eye, were traced frame by frame from the magnified image given by the stop-motion projector, for sequences selected for maximum definition of the eye and when the bird's head was still. Diameters of the outer edge of the iris and of the pupil were measured both horizontally and vertically. Pupil measures were only accepted when the measures of the outer edge remained essentially constant, when measured changes of pupil size could not be due to changes in distance of the eye from the television camera.

Pupil contraction was found to start 4–5 frames before the bird's speech, which was clearly shown by the superimposed oscilloscope record of the sound track. The pupil contraction is therefore anticipatory to its speech by about 200 ms. Often contraction is nearly completed at the start of the utterance. The pupil diameter, both horizontal and vertical, shrinks by up to half the resting diameter. Contraction seems to occur for all utterances except for (innate?) very short squawks, present on sound tracks only for single frames of the film and so for less than 40 ms duration. Contractions are shown in the graph (Fig. 1) and in the sequence of cine frames from video tape (Fig. 2).

Pupil changes associated with our utterances were more variable, and by no means always present. They seemed to occur only when the parrot was 'attending'—gazing at us with a single steady eye—and only when we uttered one of her favoured words or sounds. Some of these are her name, "Seraphita"; "Hello"; "Goodbye"; "Good boy"; "Arrio" (a Spanish exclamation);

"Pretty boy"; "Prue"; and other names of her intimate acquaintance. She also has a very human laugh and an accurate telephonebell sound. The pupils contract most dramatically for what can only be described as a 'wolf whistle'. All these are uttered spontaneously, and they may be evoked by saying them to her. Most recently, we find that she can develop a new call within seconds of hearing a new sound. She produced an accurate copy of the distinctive whir of a motor-driven Nikon camera, immediately after its first brief run, when we were trying to capture her pupil closure with high resolution directly on film. This 'Nikon whir' call was spontaneously repeated several times.

A younger male blue-fronted Amazon (Amazona aestiva) was introduced into a neighbouring cage a year ago. This parrot does not talk. His pupils are found to remain of constant size—except occasionally when he picks up an unfamiliar object when a small contraction may take place. This has also occasionally been observed for Seraphita. So pupil contraction seems to be mainly though not quite exclusively associated with utterance, attending to certain familiar utterances and sounds and also sometimes, we are inclined to believe, to internal 'rehearsal'.

We have so far no theory to account for these phenomena; neither do we know how general they may be among talking birds. Could they be signals to command other birds' attention? The contraction of the jet black pupils surrounded by their brilliant gold irises is distinct enough for this. Are they associated with difficult tasks, including handling unfamiliar objects, when depth of field may be increased and perhaps also the lens accommodated for near precise vision? Or is this phenomenon no more than (in two senses?) a case of neural cross talk?

We conclude that talking parrots can be and have apt pupils but we cannot guess what they are apt to do.

We thank Philip Clark, Tony Makepeace, Anthony Downing and Freja Gregory for technical assistance.

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Walls, G. L., The Vertebrate Eye and its Adaptive Radiation, 645-647 (Hafner, New York and London, 1963).

# Audiofrequency vibrations and gravity-wave detectors

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Non-elastic audiofrequency vibrational modes in aluminium and other metals are suggested as a possible reason for the differences reported by various observers using large aluminium alloy bars (or disks) as gravity-wave detectors. Loading of suspended gravity-wave detector bars resulting from the gravitational attraction of the Earth is also sufficient to produce the small stresses required for the self-generation of audiofrequency sounds (acoustic emission) in materials. Both the non-elastic mode response and acoustic emission frequencies depend critically on the exact microstructure and/or residual stress state of a bar.

GRAVITY-wave detectors dependent on the excitation of elastic-wave vibrational modes in a solid have been cited variously as confirming<sup>1,2</sup> or not confirming<sup>3-6</sup> the existence of gravity waves. Most of these detectors consist of long bars of an aluminium alloy (exact alloy often unspecified) with

lengths chosen to give the first or fundamental longitudinal elastic mode of vibration in the audiofrequency range. The longitudinal modes of elastic vibrations in a free-free bar or rod have frequencies,

$$v_n(elastic) = nc_1/2l = (n/2l)(E/\rho)^{1/2}...$$
 (1)

where  $n = 1, 2, 3 \dots$ , l is the length of the rod,  $c_1$  is the longitudinal sound velocity in the rod, E is the longitudinal (Young's) modulus of the rod, and  $\rho$  is the density of the rod material. Longitudinal sound velocities in aluminium alloys range from about 4.5 to  $6.4 \times 10^5$  cm s<sup>-1</sup> so that rod lengths of 153 and 366 cm have been used for detectors with first-mode frequencies (n = 1) of 1,661 and 710 Hz, for example 1.2.5.

There are also non-elastic audiofrequency vibrational modes which depend critically on the exact microstructure or "texture" of a given sample, but are independent of elastic-wave velocities and macroscopic sample dimensions<sup>10,11</sup>. That is, non-elastic mode frequencies,  $v_{kq}$ , corresponding to an ordered arrangement of atomic masses,  $m_k$ , with spacings,  $d_k$ , extending over a

distance,  $S = Nd_k$ , in the material are given to a good approximation by

$$v_{kq}$$
 (non-elastic)  $\simeq (h/8m_kS^2)q^2$  (2)

where h is Planck's constant, and  $q = 1, 2, 3 \dots (N-1)$ . For these non-elastic modes to be observed in small-amplitude vibration experiments it is also necessary that some residual or applied steady stresses be present in the material to activate the modes. Characteristic lengths, S, are determined by the mosaic patterns in single crystals and the grain or subgrain diameters in polycrystalline materials. Such lengths are most often in the micrometre range<sup>12</sup>.

#### Vibrations in aluminium

For aluminium a value of  $S = 1.07 \times 10^{-4}$  cm gives a first-mode frequency of 1,660 Hz from equation (2);  $S = 2.14 \times 10^{-4}$  cm corresponds to a first-mode frequency of 415 Hz and a second-mode frequency of 1,660 Hz. That result is particularly interesting since Hiedenreich13 has used electron-microscope transmission to show that polycrystalline aluminium, heavily worked at room temperature, consists of slightly misoriented domains about 2×10<sup>-4</sup> cm across. Beck and Hiu14 confirmed these results, and Kellar, Hirsch and Thorp<sup>16</sup> used an X-ray microbeam method to show that pure aluminium worked at room temperature first had domains about 3.6×10<sup>-1</sup> cm wide which decreased to 2.2×10<sup>-1</sup> cm after prolonged resting at room temperature. The low order non-elastic mode frequencies given by equation (2) are very sensitive to the exact value of S; values of S, in turn, vary according to the thermal and stress history (quenching, cold-working, annealing, and so on) and states of a particular piece of a given material.

From equations (1) and (2) we see that identical mode frequencies for the two types of wave correspond to vastly different wavelengths. An elastic first-mode frequency of 1,660 Hz in aluminium corresponds to a wavelength of about 300 cm and the first non-elastic mode at the same frequency has a wavelength of  $4.28 \times 10^{-4}$  cm. Thus the usual piezoelectric crystal vibration detectors respond to the acceleration of some portion of a sample surface with dimensions much less than a wavelength (elastic mode) or much greater than a wavelength (non-elastic mode) at low audiofrequencies. In addition elastic mode frequencies for a bar of fixed length, I, increase as 1,2,3,4... whereas non-elastic mode frequencies for a given microstructural length, S, increase as 1,4,9,16...; a particular bar has only one macroscopic length, but may have a microstructure with several predominant characteristic lengths or a distribution of lengths about some mean value.

Experimental evidence of non-elastic audiofrequency modes (at room temperatures) for dynamic shear compliance measurements on small metal disks was first reported16 in 1957. Some of the differences in mechanical vibrational spectra found in samples formed from the same 0.25-inch diameter extruded bar of 2S aluminium are shown in Fig. 1 where the variation of complex shear compliance,  $J^* = J' - iJ''$ , with applied vibration frequency from 100 to 2,000 Hz is adduced for three samples. All sample disks were cut from the same bar and were of virtually identical dimensions. The first sample disks (a) have a large resonance near 1,040 Hz (actually a triplet with mode frequencies 1,040, 1,060, and 1,070 Hz). Smaller resonances appear at 1,355, 1,655, and 1,860 Hz. Other resonances, not shown in Fig. 1, were observed at 2,010, 2,370 and 2,520 Hz; 2,930, 3,260, and 3,480 Hz. The groups of triplet resonances are roughly in the ratios 16:25:36:49 so that first, second and third-order modes near 66, 265, and 595 Hz might be inferred; none of these, however, appears in the data for this first sample. The spectrum of the second set of disks (Fig. 1b) is quite different, with only small resonances at 250 and 970 Hz. The third sample (Fig. 1c) again displays a very small resonance at 265 Hz, a triplet at 970, 1,000 and 1,050 Hz, but no other dispersions below 2,000 Hz.

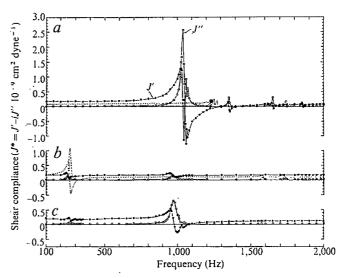


Fig. 1 Variation of complex shear compliance  $(J^* = J' - iJ'')$  with applied vibration frequency for three sets of small sample disks of 2S aluminium cut from the same extruded bar and of identical dimensions as described in the text. Dotted and dashed lines show measured values of J' and J'' respectively after No. 1 samples (a) were annealed at 1,100° F for two hours and after No. 2 samples (b) were compressed 3.0% in the direction of their axes. J' is the ratio of the in-phase component of resulting strain amplitude to the applied vibrating stress amplitude  $(a'/s_0)$  while J'' is the ratio of the 90° phase component of strain amplitude to the stress amplitude  $(a''/s_0)$  at each frequency. All measurements were made at room temperature  $(24 \pm 1^{\circ} \text{ C})$  using the method and measuring apparatus described in ref. 17, and extended from March 15 to May 14, 1962. Differences in measured compliances are attributed to variations in sample microstructures and stress states as discussed in text.

#### Cause of observed variations

These differences are, of course, attributed to differences in microstructure and/or residual stresses which vary from place to place in a material. Much larger differences in microstructures and stresses are to be expected between (and within) large bars from 5 to 10 feet long and weighing thousands of pounds such as those being used for gravity-wave detection.

Thermal and mechanical stress effects on the shear compliance spectra are demonstrated by the dotted and dashed lines (for J' and J'', respectively) in the groups of curves a and b in Fig. 1. These lines for the first sample (a) represent the results found after the sample was removed, annealed at 1,100° F for 2 h, found to have the original dimensions within  $\pm 0.0001$  inch, and then replaced in the measuring apparatus. Resonances above 2,000 Hz except the one at 2,920 Hz (moved to 2.930 Hz) also disappeared from the spectrum of this sample after annealing. By contrast the lines for the second sample (Fig. 1b) show the results found after this sample was removed and subjected to a 1,000-pound compressive stress on its parallel faces for one minute. The (mean) thickness decreased from 0.200 to 0.194 inch (3.0%) and the diameter increased as a result of this loading (major axis 0.2558, minor axis 0.2555). In addition to the large increase in the resonance at 260 Hz and the other changes shown in Fig. 1, the large resonance at 2,910 Hz 'moved' to 3,090 Hz after this load treatment.

The effects of annealing on the mechanical spectrum of the first set of 2S aluminium samples (Fig. 1a) are entirely similar to those previously reported for lead disks (99.9998 % purity).

#### 'Relevance to 'gravity-wave' observations

This discussion of the occurrence of non-elastic vibrational modes in materials is presented as a possible explanation for the differences found among various gravity-wave detectors; a particular aluminium alloy bar designed to be sensitive to vibrations at an elastic-mode frequency may, in fact, be responsive to an intrinsic, non-elastic microstructural mode

at the same (or nearly the same) frequency. Another bar of the same chemical composition and dimensions, even if cast or formed under supposedly identical conditions, may, because of slightly different thermal and/or stress histories or states, have a different microstructure and/or (mean) residual stress state which make it less responsive to the non-elastic vibrational mode frequency or frequencies found in the first bar.

A second possible explanation of the differences is that stress-induced acoustic emission (at particular, discrete frequencies) could be present and detected in some of the bars, but not in others. The same wave-mechanical theory of deformation<sup>10,11</sup> which describes the non-elastic vibrational modes previously cited also predicts the occurrence of smallamplitude audiofrequency vibrations during plastic deformation. An approximate expression for the net translational oscillating force on a lattice segment of length S in a material during steady loading is given by<sup>11</sup>

$$f_q(t) \simeq (B_1 h / Sd_k) q \exp(-i2\pi v_{kq} t)$$
 (3)

where h is Planck's constant,  $d_k$  is the regular spacing between atoms of mass  $m_k$ , extending over a distance  $S = Nd_k$ ,  $B_1$  is a velocity constant depending on the load magnitude (the macroscopic stress) and the oscillation frequency,  $v_{ky}$ , is given by equation (2), where q = 1,3,5... An estimate<sup>11</sup> of the mean force amplitude in equation (1) for q = 1 and  $B_1 = 10^3$  cm s<sup>-1</sup> is about  $2 \times 10^{-8}$  dyne cm<sup>-1</sup>. An oscillating force on the individual ordered segments of micrometre length within materials will tend to set into vibration (portions of) the entire sample; these sample vibrations will ordinariy be of very small amplitude unless a number of individual segment vibrations occur simultaneously throughout some appreciable volume of the sample. Contributions from even modes are absent from the net translational forces on segments (q =1,3,5,7..., equation (3)), but even modes do produce net oscillating moments on the lattice segments11 with amplitudes  $(B_1h/2d_k)q$  for q = 2,4,6...

The occurrence of audible sounds during severe plastic deformation is, of course, well known<sup>17,18</sup>. Of more interest to the present discussion of gravity-wave detectors, however, is the occurrence of much fainter sounds at very low stress (and strain) levels, continuing through yield as reported by Kaiser<sup>19</sup>. Sensitive piezoelectric crystal detectors similar to those used on the gravity-wave detector bars, and a very quiet loading system, were used by Kaiser to study audiofrequency sounds emanating from polycrystalline zinc, steel, aluminium, copper, lead and wood specimens loaded in tension.

An extensive investigation of acoustic emission has been carried on since 1954 by Schofield<sup>20-22</sup> over a range of stress levels, and on both single crystals and polycrystals of gold, zinc, aluminium, lead, cast tin, carbon steel and 24ST-4 aluminium. A chief conclusion reached by Schofield is that acoustic emission consists of two distinct types: (1) random, low frequency, high amplitude bursts or pulses at low stresses; and (2) a continuous high frequency emission of initial low amplitude which increases in amplitude with increasing applied stress. Schofield also found that acoustic pulses are of extremely low energy, and amplifications of 106 to 107 are required for the detection of individual pulses. In some deformation processes avalanches of pulses may occur to produce a combined signal of much more energy.

Thus the possibility of acoustic emissions must be considered in connection with the large aluminium (or aluminium alloy) bars used for gravity-wave detectors. Many of these bars are suspended from their midpoints by steel cables or chains. Piezoelectric vibration detectors are usually placed around the bar circumference at the mid-point or sometimes on the ends. A distributed load resulting from the Earth's gravitational attraction acts on such a suspended bar of cross-sectional area A and length I with a value per unit length, F/I, =  $\rho gA$ , where  $\rho$  is the density and g is the gravitational acceleration. Bending moments around the plane of suspension will also

be present, and at the cross-sectional plane of suspension a shear stress of pgl exists. For a 2024-T4 aluminium bar of length 150 cm and diameter 19 cm, for example, this shear stress amounts to  $4.06 \times 10^5$  dyne cm<sup>-2</sup> (5.82 pounds inch<sup>-2</sup>). In addition, the bending moments will produce tension at the top of the suspension plane and compression at its bottom with mean stress values of around  $1.5 \times 10^7$  dyne cm<sup>-2</sup>; exact calculations of the stress distribution can be made, but are not needed to demonstrate that the 'gravitational' stress levels in the bars are sufficient to produce acoustic emission at the rate of several pulses per second. Further, and of particular interest regarding gravity-wave detectors, Schofield observed in 1958 (refs 20-22):

"... The influence of the material from the microphysical standpoint appears to be a prominent factor determining the characteristics of the emission signals. Although the specimens were prepared from the same stock and are assumed to be 'identical' from the engineering viewpoint, the emission sources, if originating at the microlevel in the material, would be affected by the actual metallurgical and physical differences occurring locally . . . ".

As suggested by equation (3) and confirmed by these experimental observations, bars of identical dimensions, chemical composition, and nominal structures may nevertheless 'emit' somewhat different audiofrequency vibrations under the same load because of differences in microstructures or initial stress states. In particular a narrow band or single-frequency vibration detector on one bar may record no signals because it is not tuned to any of the non-elastic mode frequencies corresponding to the characteristic microstructural length (or lengths), S, of that bar. A second bar, however, may have a non-elastic mode frequency identical to that of the detector frequency and display some of the audiofrequency bursts or 'events' which occur at very low stress levels.

Elaborate precautions taken to isolate the detector systems from ambient mechanical and sound vibration, shielding from electromagnetic radiation and so on, are all of little avail if possible vibrations generated within a detector bar are ignored.

Thus expectant observers of gravitational radiation should take into account their bar material microstates and the non-elastic vibration phenomena cited here.

I thank Leon Madansky and Stanley Corrsin for discussions.

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# Palaeomagnetic evidence shows Malay Peninsula was not a part of Gondwanaland

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Palaeomagnetic results from Malaya show that the Malay Peninsula lay at 15° N during the late Palaeozoic, a result incompatible with the hypothesis that it once formed a part of Gondwanaland adjacent to India or Australia. Cretaceous results suggest the Peninsula was not at that time finally welded to the Asian mainland.

DEBATE has recently centred around the proposition that parts of South-east Asia were once a part of Gondwanaland. Burton¹ tried to explain the westerly origin of the sediments of northwest Malaya by supposing, in a reconstruction of Gondwanaland, that the west coast of the Malay Peninsula lay adjacent to

the east coast of India. His argument was supported by stratigraphic comparisons and the reasonably good fit of the coast-lines. Ridd² went one step further and fitted the whole of mainland South-east Asia and the Sunda shelf region between western Australia on the east, and the east coast of India on the west. This reconstruction was subsequently accepted by Audley-Charles et al.³ who incorporated it into their hypothesis of the development of eastern Indonesia. Stauffer and Gobbett⁴, however, raised serious objections to such a reconstruction from palaeoclimatic and other evidence. During the late Palaeozoic both India⁵ and Australia⁶ suffered continental glaciation and lay in high southern latitudes⁻. By contrast the

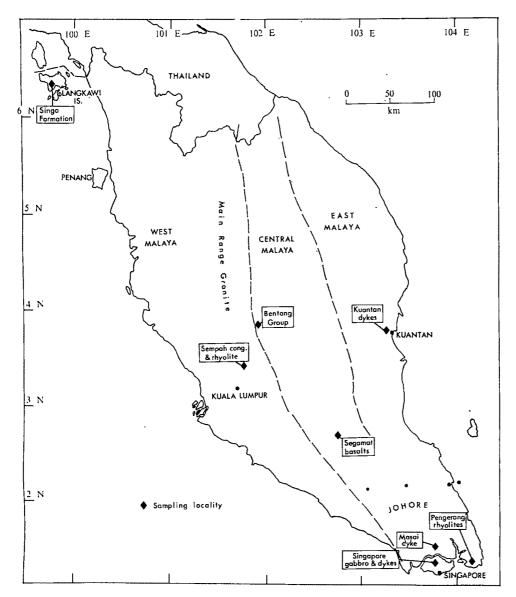


Fig. 1 Map of the Malay Peninsula showing sampling localities.

geological record of South-east Asia generally indicates tropical or subtropical conditions during the late Palaeozoic<sup>4</sup>. That the Permian of South-east Asia was laid down in warm water conditions is also accepted by Ridd<sup>8</sup>.

Both the palaeoclimatic problems and palaeogeographic reconstructions put forward can be tested using palaeomagnetic measurements. We report here the results of a reconnaissance survey of the palaeomagnetism of rocks of the Malay Peninsula. Some very preliminary results of this survey have already been reported.

Geology and sampling

The Malay Peninsula can be divided into three geologically distinct zones shown in Fig. 1. Since the first discovery of Lower Palaeozoic fossils by Jones<sup>10</sup> it has been found that Lower Palaeozoic rocks are restricted to the western zone. Recent work in Thailand<sup>11,12</sup> has recognised the extension of this western zone of Lower Palaeozoic rocks through peninsular Thailand into north-west Thailand and eastern Burma and northwards into Yunnan in China. This long geosynclinal belt has been referred to by Burton<sup>11</sup> as the Yunnan-Malay geosyncline. In the Malay Peninsula the boundary with the Lower Palaeozoic belt is approximately marked by the so-called Bentong Line<sup>13</sup>

Table 1 Analytical data for four samples of Pengerang rhyolites (total rocks)

ANU no. Rb (p.p.m.) Sr (p.p.m.) 87 Rb/867 11-699 223 38 17.23 71-700 250 35 20.58 71-702 247 120 5.96 71-703 224 134 4.82	0.7692 0.7784 0.7302
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The decay constant for <sup>87</sup>Rb used was  $1.39 \times 10^{-11}$  yr<sup>-1</sup> with statistical parameters appropriate to the experimental precision of the instrumental procedures.

ANU, Australian National University.

which includes abundant radiolarian chert, some serpentine masses, metabasites and dolerite sills. It has been suggested that this marks the former position of a late Palaeozoic subduction zone<sup>14</sup>. The central zone is characterised by folded clastic Triassic–Jurassic carbonate and continental deposits whilst the eastern zone is composed of predominantly deep-water marine clastic Upper Palaeozoic rocks with patches of Upper Mesozoic continental deposits. The central and eastern regions are together considered by Stauffer<sup>15</sup> to be an island arc formed in late Palaeozoic times and welded on to the block to the west. The most recent summary of the geology of the Malay Peninsula

is given by Gobbett and Hutchison<sup>16</sup>. In the present study hand samples, oriented by Brunton compass, were collected from a number of sedimentary and volcanic formations and igneous intrusions ranging in age from Carboniferous to Cretaceous (Fig. 1).

Six samples of red muddy sandstone from the Bentong Group were collected between mileposts 75 and 76 on the road from Kuala Lumpur to Raub (3.8°N, 101.8°E). The dip of the beds ranges from 30° to 70°, and in this region they are overlain, apparently conformably, by fossiliferous Visean shales and sandstones<sup>27</sup>. The samples are thus probably Lower Carboniferous, although they could be older.

Three samples of laminated muddy sandstone were collected from the Singa Formation on the south-west part of Langkawi Island, off the north-west coast of the Malay Peninsula (6.2°N, 99.8°E). The formation covers the whole of the Carboniferous<sup>17</sup> and the stratigraphic position of the samples suggests they lie in the upper part of the Lower Carboniferous. The beds have an easterly dip of between 25° and 30°.

Twelve samples of reddish sandy mudstone were collected from the Sempah conglomerate together with seven samples of the overlying rhyolite in the Genting Sempah area on the Ulu Kali Road (3.4°N, 101.8°E). The rocks dip east at angles between 20° and 65°. Although inconclusive, Rb-Sr dating of the rhyolites<sup>18</sup> suggests they are most probably Early Permian in age with a maximum possible age of Late Carboniferous.

Ten samples were collected from rhyolites occurring at Pengerang in south-east Johore (1.4°N, 104.2°E). The lavas dip north-west at between 19° and 36°. Rb-Sr measurements on these samples suggest they are Early Triassic or Permo-Triassic in age. This is identical with the age of the south Johore and Singapore granites<sup>18</sup>, with which they are probably associated.

Ten samples of basalt and two of red agglomerate were collected from the Segamat basalts of Negri Sembilan (2.5°N, 102.8°E) described by Grubb¹³. The lavas lie unconformably on Middle to Upper Triassic rocks. The flows appear to dip in a south-westerly direction at 15°, although this dip may be in part original. A single, whole rock, K-Ar age determination from these basalts gives an age of 62 Myr (ref. 18) but this is regarded as a minimum age in view of demonstrated argon loss from other rocks in the region¹³. The basalts are probably Cretaceous in age.

Three samples were collected from basaltic dykes near Kuantan, Pahang (3.8°N, 103.3°E) and two from a dyke at Masai, Johore (1.5°N, 103.8°E). One of the Kuantan dykes gives a K-Ar age of 110 Myr and is therefore probably Cretaceous in age<sup>18</sup>.

One sample of gabbro and four samples, one from each of four thin dykes intruding the gabbro, were collected from two quarries on Singapore Island (1.3°N, 103.8°E). The gabbro is intruded by the Singapore granite of Early Triassic age<sup>18</sup> and basic dykes are not known to cut the granite. The dykes are

Rock unit	Age	n	D	I	R	k	$a_{95}$
Segamat basalts, Kuantan and Massai dykes	K	16	136	-31	15.081	16.3	9.4
Pengerang rhyolites	(Pu-)Trl	10	206	-39	9.638	24.9	9.9
Sempah conglomerate and rhyolites	(Cu-)Pl	14	212	-32	13.083	14.1	10.9
Bentong Group and Singa formation	C(!)	8	213	16	7.725	25.4	11.1
Singapore gabbro and dykes	>Trl	5	194	<b>-</b> 21	4.758	16.5	19.3

n, No. of samples; D, declination east of true north; I, inclination-positive downwards; R, resultant of the n unit vectors; k, precision parameter;  $\alpha_{95}$ , radius of circle of 95% confidence about the mean.

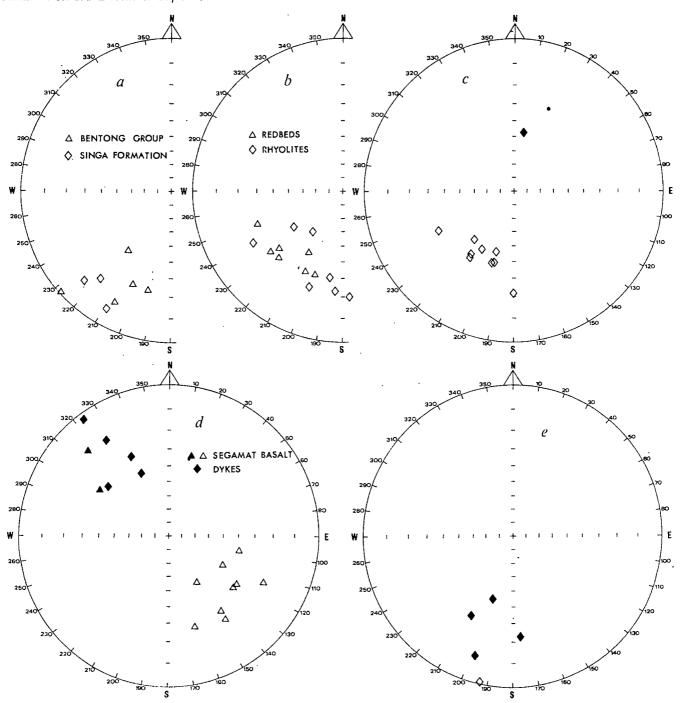


Fig. 2 Stereographic projections showing the cleaned directions of magnetisation for (a) Bentong Group and Singa Formation, (b) Sempah conglomerate and rhyolite, (c) Pengerang rhyolites (d) Segamat basalts, Kuantan and Masai dykes, (e) Singapore gabbro and dykes. Solid symbols on the lower hemisphere, open symbols on the upper hemisphere.

Several specimens were cored and sliced from each hand sample and the natural remanent magnetisation was measured using a PAR-SM1 magnetometer. Pilot specimens were subjected to alternating field demagnetisation in increasing fields<sup>20</sup>. Most of these measurements were made at the Australian National University but during the final stages of this survey some additional measurements were made in the newly established palaeomagnetic laboratory in Kuala Lumpur. Measurements were then made on a Digico magnetometer and magnetic cleaning undertaken with a Schonstedt demagnetiser. In Canberra, pilot specimens of the sedimentary samples were also subjected to stepwise thermal demagnetisation in which the specimens were heated and cooled in a magnetic field of one γ (1nT) or less<sup>21</sup>.

probably of similar age to the gabbro which must be older than Early Triassic, but by how much is unknown, as there is no evidence which could define a maximum age.

#### Rb-Sr age of the Pengerang rhyolites

Four samples of Pengerang rhyolites were analysed as total rock samples at the Australian National University by the Rb-Sr method (see Table 1). The individual analyses show inadequate enrichment in radiogenic strontium to make reliable estimates

of age. A statistical analysis using the least-squares method with a two-error regression indicates an age of 238  $\pm$  23 Myr. The high mean square of weighted deviates (18.37) places this estimate in the category of a reconnaissance age determination, in so far as the samples probably had slightly different initial \*Sr/86Sr ratios; but the age can reliably be placed close to the Permo-Triassic boundary.

Bignell<sup>18</sup> obtained an isochron of  $225 \pm 6$  Myr for a combination of three analyses of Singapore granite and two of

Rock unit	Age	n		Directi	ions		Poles	
	_		$k_{b}$	$k_a$	Sig.	$K_{b}$	$K_{a}$	Sig.
Segamat basalts, Kuantan and Masai dykes	к	16	18.5	16.3	No	22.3	18.6	No
Pengerang rhyolites	(Pu-)Tr1	10	13.9	24.9	No	9.4	21.5	Yes
Sempah conglomerate and rhyolites	(Cu-)P1	14	10.9	14.1	No	6.8	13.4	Yes
Bentong Group and Singa formation	C(1)	8	9.7	25.4	Yes	17.3	37.0	No

 $k_b$   $K_b$  are precision parameters before applying tilt corrections and  $k_a$   $K_a$  are those after applying tilt corrections.

Johore granites, using a decay constant of  $1.47 \times 10^{-11}$  yr<sup>-1</sup>. Converted to the 1.39 decay constant used in our analysis, this result gives a figure of  $238 \pm 6$  Myr. Although this is also in the category of a reconnaissance result as the samples are from different localities, it is identical with that for the Pengerang rhyolites, which may provisionally be regarded as the effusive equivalent of the granites.

#### Palaeomagnetic results

Samples from the Bentong Group and Singa Formation responded well to thermal cleaning. Pilot specimens showed excellent stability up to the Curie Point of haematite. A small viscous component in the present field direction was removed at 300° C and on correcting for the dip of the strata, the samples group in a south-westerly direction with shallow inclinations (Fig. 2a). One sample was too weakly magnetised and its remanence could not be measured.

There was no difference in response of the red sediments from the Sempah conglomerate to either thermal or magnetic cleaning. Both the red sediments and rhyolite were therefore cleaned at 30 mT. Five of the twelve samples of red sediment, however, remained clustered about the present field direction, could not be cleaned and were rejected. This illustrates a problem associated with sampling red sediments in tropical countries. Even if samples are collected from road cuttings within a few years of exposure, weathering can have progressed fairly deep. In the case of red sediments it is sometimes very difficult to distinguish whether substantial deep weathering has occurred. Seven samples of red sandstone and seven rhyolites gave consistent directions after cleaning, similar to those given by the Bentong Group and Singa formations (Fig. 2b). All these late Palaeozoic samples are reverse magnetised.

The Pengerang rhyolites were extremely stable to alternating field demagnetisation, and blanket cleaning at 30 mT was carried out. One of the ten samples was normally magnetised and the other nine are reversed. The mean direction (Table 2, Fig. 2c) is very similar to that of the other late Palaeozoic groups. The basic rocks from Singapore and Johore and at Segamat and Kuantan were less stable than the rhyolites. One of

the Segamat basalts proved to be unstable but the remainder of the samples could be cleaned at 20 mT. The Segamat flows are all reverse magnetised whilst the red agglomerate and the dykes from Masai and Kuantan are normally magnetised

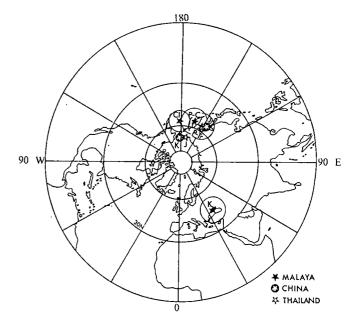


Fig. 3 Palaeomagnetic poles derived from the Malay Peninsula compared with those from China<sup>7</sup> and Thailand<sup>23</sup>.

(Fig. 2d). The Singapore gabbro and dykes are all reverse magnetised (Fig. 2e).

The mean direction of magnetisation for each of the groups is given in Table 2 together with the usual statistical parameters. The three late Palaeozoic means are very close to one another with only one of the 32 samples being normally magnetised.

Rock unit	Age	n	Lat. N	Long. E	K	$A_{95}$
Segamat basalts, Kuantan and Masai dykes	K * *	• 16 •	44	35	18.6	8.7
Pengerang rhyolites	(Pu-)Trl	10	57	152	21.5	10.7
Sempah conglomerate and rhyolites	(Cu-)Pl	14	55	164	13.4	11.2
Bentong Group and Singa formation	C(I)	8	57	182	37.0	9.2

n, No. of samples; K, precision parameter;  $A_{95}$ , radius of circle of 95% confidence about mean pole position.

Because of the different attitudes of the beds sampled, substantial tilt corrections have been applied. The statistical significance of the application of the fold test to four of the groups is given in Table 3 using the tables of McElhinny<sup>22</sup>. In spite of the relatively small collection of samples, there is a significant fold test for either the directions or poles for three of the four groups. This argues strongly for the stability of magnetisation of the samples at least since the time of folding, generally regarded as Late Triassic. The tilt corrections are too small for the Cretaceous group to produce any positive fold test. The presence of roughly opposed directions of magnetisation (Fig. 3d), however, provides additional stability evidence.

#### Palaeomagnetic poles and palaeogeography

Palaeomagnetic pole positions for four of the age groups are listed in Table 4. The five samples from Singapore are not considered sufficient to calculate a viable pole position and, in any case, the precise age of these rocks is not known. Between the Early Carboniferous and Early Triassic there appears to be a small westerly migration of the pole but it is hardly significant (Fig. 3). In fact, the data are such that it might be valid to combine all the 32 late Palaeozoic measurements together to

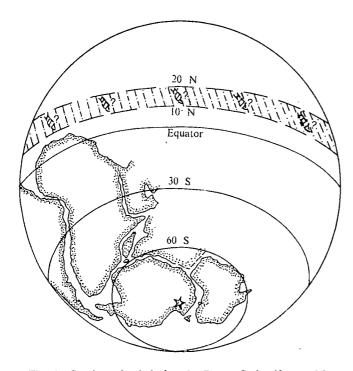


Fig. 4 Gondwanaland during the Permo-Carboniferous. The star is the mean palaeomagnetic south pole24. The Malay Peninsula must lie at 15°N, anywhere along the arc of a circle 105° from the pole. A 10° wide belt is shown together with some possible positions of the Peninsula.

produce an overall pole at 56°N, 165°E with  $A_{95} = 6.3$ °. The distribution of these poles changes from K = 6.1 to K = 17.1after correcting for the attitude of the beds, a change that is statistically highly significant<sup>22</sup>.

The palaeomagnetic poles are plotted in Fig. 3 where they are compared with poles from the Jurassic of Thailand<sup>23</sup> and the Jurassic and Cretaceous of China7. These lie fairly close to the Malayan late Palaeozoic poles but are well removed from the Malayan Cretaceous pole. This would suggest that the Malay Peninsula was not firmly attached to the mainland in Cretaceous times. The magnetic inclinations observed during the late Palaeozoic and Cretaceous in Malaya are very similar (Table 2) indicating that the Peninsula continuously occupied low latitudes of about 15°N during this time. This strongly supports the contention4 that the Malay Peninsula occupied low latitudes during the late Palaeozoic. Between the Permo-Triassic and the Cretaceous, the Peninsula underwent a clockwise rotation of 70° whilst maintaining much the same palaeolatitude.

The data for the late Palaeozoic may be used to test the hypothesis that the Malay Peninsula once formed a part of Gondwanaland. Since it is known that the late Palaeozoic is a period of predominantly reversed polarity<sup>7</sup> the data of Table 2 indicate that the Peninsula lay 105° away from the South Pole, that is, in the northern hemisphere at 15°N. Using the latest estimate of the Permo-Carboniferous pole with respect to Gondwanaland<sup>24</sup>, the Malay Peninsula must lie along a circle lying 105° away from this pole (Fig. 4). The palaeomagnetic data are clearly inconsistent with a position adjacent to India or Australia in the late Palaeozoic. Since Gondwanaland did not break up until the Jurassic, it seems unlikely that the Malay Peninsula was associated with the supercontinent in the early Palaeozoic and broke away before the other segments.

From Fig. 4 it can be seen that with due allowance for possible inaccuracies inherent in the method of positioning the Peninsula and Gondwanaland, the data do not completely rule out the hypothesis of Stauffer<sup>15</sup> that the Malay Peninsula may have been adjacent to parts of north Africa in the Palaeozoic. Further palaeomagnetic work planned for the Lower Palaeozoic of the Malay Peninsula may throw more light on this hypothesis.

Samples from the Singa Formation were collected by Dr P. H. Stauffer. Discussions with Drs C. S. Hutchison, P. H. Stauffer and T. E. Yancey were helpful. D. Edwards and P. Schmidt assisted with some of the magnetic measurements.

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# Structural homology of myosin alkali light chains, troponin C and carp calcium binding protein

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Sequence comparison between the myosin alkali light chain, troponin C and carp calcium binding protein shows that the pattern of hydrophobic residues which forms the core of calcium binding protein is preserved in both troponin C and myosin alkali light chain. Strong structural similarities between the proteins are suggested. The role of gene duplication in the evolution of troponin C and myosin alkali light chain is discussed.

An understanding of the mechanism of muscle contraction at the molecular level requires knowledge of the structure of the proteins involved and the ways in which they interact. The only muscle protein whose three-dimensional structure is known is a calcium binding protein from carp (CBP), often called parvalbumin<sup>1,2</sup>. The function of CBP is unknown, but the amino acid sequence of the calcium binding component of rabbit troponin, troponin C (TNC)<sup>3</sup> is closely related<sup>4,8</sup> Collins<sup>6</sup> has observed a sequence homology between TNC and parts of the alkali light chains (ALC) of rabbit myosin<sup>7,8</sup>. We have made a more searching comparison between these proteins using the completed light chain sequence<sup>8</sup> to see how far the structural features of CBP are preserved in TNC and ALC. One aim was to try to discover why the free alkali light chains do not bind calcium and to account for the difference between the strong and weak calcium binding sites of TNC10. Another was to investigate the role of gene duplication in the evolution of these molecules<sup>1,11</sup>. Finally the general interest in the role of light chains in the function of myosin makes these comparisons pertinent.

#### Structural considerations

The characteristic features of CBP, established from the X-ray diffraction studies of Kretsinger<sup>2</sup>, may be summarised as follows. The protein contains two sites for binding calcium, related by a twofold symmetry axis. Each site lies in a corner between two helical regions, giving a basic structural unit; helix A-calcium site-helix B (abbreviated A-S-B). Duplication of this unit produces a two-unit structure (I-II) of the type A1-S1-B1-L-A2-S2-B2 (where L is a link region). CBP contains two additional helices at its N-terminal end and the packing of the six helices produces a hydrophobic core.

Figure 1a illustrates the structural features of carp CBP. Residues circled are buried in the core of the molecule<sup>2</sup> or make important hydrophobic contacts, and lines mark the main van der Waals' interactions. Figure 1a shows the pronounced asymmetry in the contacts between the four helices, such that the patterns of apolar groups are characteristically different. (In discussing these structures, a structural notation is used in which [A1(8)] refers to position 8 of helix A1.)

Ile [S(8)], (position 8 of the calcium site) is important structurally because of its buried side chain and a hydrogen bond linking the two adjacent Ile [S(8)] residues<sup>2</sup>. Phe [S1(7)] may screen calcium site 1 from water, whereas site 2 has a lysine residue at [S2(7)] and may be more exposed. The amino acids which coordinate to calcium (Fig 1a) are listed in Table 1.

Important structural features in the link region are the salt bridge between Arg [L12(5)] and Glu [A2(3)], in a crevice between helices B1 and A2, and two apolar interactions between Leu [B1(6)] and the main chain of Arg [L12(5)], and between Leu [12(7)] and Thr [A2(4)]. The internal residues Leu [B1(8)] and Val [B2(8)] both appear to interact with the two N-terminal helices. If the structure of TNC reflects that of CBP, these positions might form part of the hydrophobic core in contact with the structural units III and IV.

#### Sequence comparisons

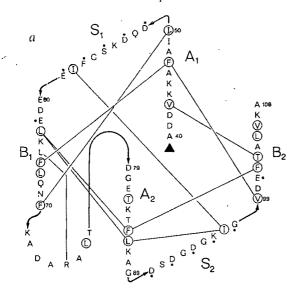
Comparison of TNC with CBP immediately suggested<sup>3</sup> that troponin C contains four units of the type A-S-B. This structure could arise by further duplication of the calcium binding components of CBP to produce a tetrahedral molecule of the form: (A1-S1-B1-L12-A2-S2-B2)-J23-(A3-S3-B3-L34-A4-S4-B4), with a new joining piece J23. Using the complete sequence of the alkali 2 light chain we have aligned the sequences using a computer program which compared sequences span by span, with a span size of 11 residues<sup>12</sup>. Similarities between amino acids were scored according to both their chemical similarity and the frequency of substitutions observed between them in a large number of different proteins. This procedure gives high scores for conservative replacements which fit readily into the same three-dimensional structure. The sequences of both TNC and ALC were compared with themselves as well as with each other, in order to identify internal repeats. Figure 1b and c shows the sequences drawn out to follow the structural features of CBP (compare Fig. 1a).

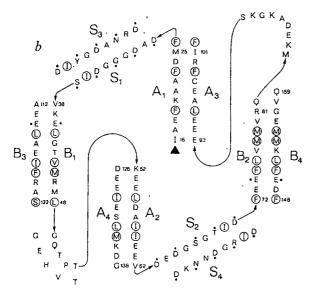
Table I Calcium co	ordinating	groups in	the calcium	i binding pro	tein, tropo	nin Cana e	quivalent p	ositions in t	he alkalı li	ght chains	
	СВ	P		TT	iC			AL	С		_
Coordinating position	$S_1$	$S_2$	$S_1$	$S_2$	$S_3$	S <sub>4</sub>	$S_1$	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	
$S_{(1)}$ $X$	Asp	Asp	<ul><li>Asp</li></ul>	Asp	Asp	Asp	Asp	Asp	Asp	Ğİn	
$S_{(3)}$ Y	Asp	Asp	Asp	Asp *	Asn	Asn	Thr	Gln	Glu	Asn	

Coordinating position	$\mathbf{S_1}$	$S_2$	$S_1$	$S_2$	$S_3$	S <sub>4</sub>	$S_1$	$S_2$	$S_{a}$	S <sub>4</sub>
$S_{(1)}$ $X$	Asp	Asp	<ul><li>Asp</li></ul>	Asp	Asp	Asp	Asp	Asp	Asp	Ğln
$S_{(3)}$ Y	Asp	Asp	Asp	Asp •	Asn	Asn	Thr	Gln	Glu	Asp
$S_{(5)}$ Z	Ser	Asp	Gly	Ser	Asp	Asp	Asp	Asn	Gly	Asn
$S_{(7)} - Y$	Phet	Lys†	Asp	Thr	Tyr	Arg	Lys	Lys	Val	Cys
$S_{(9)}$ $-X$	Glu	Gly*	Ser	Asp	Asp	Asp	Thr	Glu	Met	Asn
$\mathbf{B}_{(3)}$ $-\mathbf{Z}$	Glu	Glu	Glu	Glu	Glu	Glu	Ğln	Gln	Glu	Ala
Negative charges	4	4	4	4	4	4	2	2	3	1

X, Y and Z refer to the octahedral vertices of the calcium ligating positions, with the sequence positions indicated.
\*In CBP the Gly at position (-X) does not bind calcium, but the Asp at position (Y) links in both the Y and -X coordinating directions.

†In the (-Y) position, the carbonyl oxygen of the peptide bond coordinates to calcium.





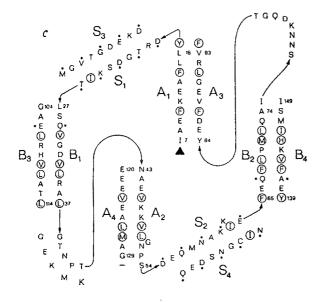


Fig. 1 a, Amino acid sequence of the two calcium binding structural units of carp calcium binding protein. Symbols AI, BI and so on are the helical segments, while SI and S2 denote the calcium binding sites. Residues circled are buried hydrophobic groups<sup>2</sup> or those making important hydrophobic contacts, and

The alignment of TNC with ALC is particularly good in the following regions: helix A1, the middle of helix B1 to L12 and the start of A2, A3, A4 and also the second half of S4 to the end of B4. As each sequence is repetitive there are also many cross-correlations, for example between TNC [L12] and ALC [L34] or vice versa.

The most striking observation is the preservation in both TNC and ALC of the pattern of hydrophobic residues which form the core of CBP. Table 2 summarises the data from Fig. 1, and shows that virtually every substitution is conservative. This suggests strong structural similarities between the three proteins. We have built a molecular model from Kretsinger's coordinates<sup>2</sup> to check that all the substitutions are feasible. Another residue which is conserved is the Glu at [A2(3)] and [A4(3)]. This is the position of the salt bridge in CBP, but the Arg in the link region is not present in either TNC or ALC. Link L34 of ALC contains two lysine residues, however, one of which might substitute in the salt bridge, while helices B1 and B3 of TNC and B1 of ALC contain arginine at position 9 which might preserve this bridge.

The only change required to fit both TNC and ALC to the CBP structure is to shorten the link region between helices B1 and A2, and B3 and A4. These links appear unnecessarily convoluted in the CBP model, and Pro [L12(4)] in both TNC and ALC could mark a bend which shortens this link. Also the glycine at the beginning of this region in both proteins could provide more flexibility at the start of the corner.

#### Internal repeats in TNC and ALC

Comparison of the sequence of TNC with itself shows clear evidence for internal repeats between structural units (I-II) and (III-IV), with unit I like III and II like IV, as observed by Collins et al.<sup>3</sup>. Helical regions which match particularly well are A2 with B4 (Fig. 1b). Such homologies are much stronger than those seen in normal proteins<sup>12</sup> and support the hypothesis of gene duplication in the evolution of this molecule. In ALC, the repeats follow a similar pattern but are much weaker. A surprising similarity which is not consistent with this pattern is the striking homology between A3 and B3 in TNC, which is analogous to that observed between helices A2 and B2 in CBP<sup>11</sup>. Again there are similar homologies in ALC, between A2 and B3 and between A3 and B2.

#### Calcium binding sites

Each of the four calcium sites of TNC and both sites of CBP contain four acidic residues within the six coordinating positions, (Table 1). Comparison of the TNC sites shows striking similarities between S3 and S4 which contain six identical residues and conserve a basic residue at position

the lines mark the main van der Waals' interactions. \*, Residues ligated to calcium ions; A, the start of the A1 helix is at residue 40 in the sequence, which follows the direction of the arrows to the C-terminal residue 108. For simplicity it has been assumed that each helix contains exactly 11 amino acids in every case. The one letter code for amino acids has been used in all the figures, where D=Asp, E=Glu, T=Thr, S=Ser, N=Asn, Q=Gln, K=Lys, R=Arg, H=His, G=Gly, A=Ala, V=Val, M=Met, I=Ile, L=Leu, Y=Tyr, F=Phe, P=Pro, C=Cys, and W=Try. b, Amino acid sequence of rabbit troponin C drawn out to correspond to the structural features of carp CBP, but with four calcium sites and four pairs of helices. A. The start of helix A1 corresponds to residue 16 in the sequence, while the C-terminus is at residue 159. c, Amino acid sequence of rabbit alkali light chain 2 drawn out as in b. A. The start of helix A1 corresponds to residue 7 in the sequence while the C-terminus is at residue 149. (The alkali light chain 1 has an additional 41 residues at its N-terminal end\*.) Other than residues 7 and 8 at the beginning of helix A1, its sequence is identical to that shown.

S(2). The match between S1 and S2 is less good although five residues are identical.

These two matches between the pairs of calcium sites S1-S2 and S3-S4 form a different pattern from that predicted on the basis of gene duplication, that is the members of each pair are more alike than S1 to S3 or S2 to S4. When the sequence of CBP was aligned with those of TNC and ALC simultaneously, structural unit II of CBP showed close resemblance throughout to TNC (IV) and ALC (IV). This is also evident in the comparisons between the calcium coordinating residues of S2 in CBP and S4 in TNC (Table 1). The calcium sites show other quite strong similarities: the tyrosine in the -Y coordinating position of TNC [S3] is comparable with phenylalanine in CBP [S1]. S1 of TNC is like S2 of CBP in having no acid group at position -X, whereas the Asp at position Y binds to calcium in both the Y and -X coordinating position (Table 1). There are five identical residues in the comparison of these two sites, but TNC [S1] contains an aspartic acid at position -Y, which makes this site unique.

Isolated ALC does not bind calcium under physiologically significant conditions, and there are less than four negatively charged groups in every 'site' of this protein (Table 1). Examination of the molecular model indicates other changes which might reduce the affinity for calcium by distorting the binding sites. For example, helix A2 would be disrupted by the extra glycine residue following Leu [A2(8)] and proline at [A2(10)], while proline [B2(6)] would distort this helix also. Furthermore, site S2 has an extra basic residue inserted in front of Ile [S2(8)]. Similarly the presence of bulky valine and methionine residues could block site S3. Thus it is not surprising that ALC fails to bind calcium ions with high affinity even though many of the structural features of the sites are apparently preserved.

Considering the four calcium binding sites of TNC in more detail, Potter and Gergely<sup>10</sup> have recently shown that isolated TNC contains two classes of calcium binding sites with different affinities. According to the CBP structural model, sites S3 and S4 are close together on either side of the approximate twofold axis at one end of the molecule, while sites S1 and S2 are close together at the other end. Sites S3 and S4 are most like the two sites of CBP, while S1 and S2 are more similar to one another than to either S3 or S4.

The most obvious interpretation of the sequences is that S3 and S4 have evolved into particularly strong binding sites, whereas S1 and S2 are weaker ones. But the structure suggests a more interesting possibility. There is evidence that the binding of calcium at the lower affinity sites of TNC switches 'on' muscle contraction and causes a conformational change in troponin (see ref. 13 for review). This suggests that during evolution the structure of the weaker binding sites acquired special constraints

which produced a lower affinity for calcium. The mechanism could then be similar to the binding of oxygen in haemoglobin, where the affinity of the haem groups is reduced by constraints in the tense deoxy structure14. On stereochemical grounds sites S1 and S2 which each contain two or more glycine residues, should be flexible and unconstrained with a high calcium affinity, while sites S3 and S4 would be more rigid and constrained. Also by analogy with CBP, site S3 with Tyr at S(7) is less exposed to solvent than site S4, which has Arg at S(7). Furthermore Site S4 has two basic groups at positions S(2) and S(7) whereas site S3 has only one. These facts suggest that site S4 may be the last to bind calcium and the first to release it, since the basic residues might weaken the calcium affinity of this site. It should be remembered, however, that the calcium affinities are altered in the complete troponin complex.

#### Gene duplication

The hypothesis that both TNC and ALC arose by two successive duplication events, producing first a dimeric structure and then a tetrameric one, is confirmed by Collins' analysis<sup>3,6</sup>, and by our own computations. This is most clearly seen from the similarity between structural units I and III of TNC and between helices B2 and B4. The repeats are much stronger in TNC than ALC, suggesting that TNC is closer to the primitive sequence. CBP could then have exolved by adding two extra helices to the primitive dimeric unit¹ or by degradation of the tetrameric structure (I-II-III-IV) to (II-III-IV). The latter seems more likely since sites S1 and S2 of CBP closely resemble sites S3 and S4 of TNC, and the first helix of CBP is noticeably similar to the A helices of TNC. (The sequence of this helix is Asp-Ala-Ala-Ala-Ala-Leu-Glu-Ala-Cys.)

Proteins with a repeating sequence of the type X-X' and an approximate twofold axis are quite common, and probably often evolve from a structural dimer X:X. If an arbitrary structure X duplicates, it is highly improbable that the two halves of structure will fit together, and a good fit would require rapid evolution independently in the two halves leading to a structure  $X_1-X_2$ , with  $X_1$  rather different from X2. But if X evolves to form a well fitting dimer X:X before the duplication, the duplicated molecule X-X requires few modifications and the two halves can remain very similar. One suggestive example is the ferredoxin of Peptococcus aerogenes15, where the polypeptide chain has two repeat units and there are two similar iron binding sites formed by clusters of sulphydryl groups. Surprisingly each site is formed by groups from both halves of the sequence, so that dimer formation is essential for iron binding to occur. It is therefore probable that the halfchain units evolved the power to interact before duplication produced a 'single chain dimer'. Applying this principle to TNC leads to the suggestion that a primitive unstable

Helix residue no.	Helix Al			Helix A3		Helix B1			Helix B3	
	CBP	TNC	ALC	TNC	ALC	CBP	TNC	ALC	TNC	ALC
4	Val	Phe	Phe	Leu	Phe	Leu	Leu	Val	Leu	Leu
7	(Ala)	(Ala)	(Ala)	(Cys)	(Gly)	Phe	Val	Val	Ile	Val
8	Phe	Phe	Phe	Phe	Leu	Leu	Met	Leu	Phe	Leu
11	Ile	Phe	Tyr	Phe	Phe	Phe	Leu	Leu	(Ser)	Leu
		Helix A2 • •			Helix A4		Helix B2			Helix B4
	CBP	TNC	ALC	TNC*	ALC	CBP	TNC	ALC	TNC	ALC
1	(Asp)	(Lys)	(Asp)	(Asp)	(Glu)	Val	Phe	Phe	Phe	Tyr
4	(Thr)	Leu	Val	He	Val	Phe	Phe	Phe	Phe	Phe
5	(Lys)	(Asp)	(Lys)	(Glu)	(Glu)	(Thr)	Leu	Leu	Leu	Val
7 .	Phe	Ìle	Val	Leu	Leu	Leu	Mct	Met	Met	(His)
8	Leu	Ile	Leu	Met	Met	Val	Met	Leu	Met	Ile

The hydrophobic residues shown are those which play an important part in holding the helices and calcium sites together in the CBP structure. Residues in brackets are not hydrophobic, and demonstrate the asymmetry of the patterns of hydrophobic groups in the different helices.

structure of the type A-S-B was originally stabilised by formation of a dimer, and gene duplication subsequently yielded the two-unit structure (A-S-B)-L-(A-S-B). The similarity between helices A and B which has already been noted could be accounted for if the (A-S-B) unit itself was formed from a single precursor helix H and a piece of polypeptide chain, C. Dimenisation of H-C to stabilise the helices, followed by gene duplication would lead to H-C-H-C, the precursor of (A-S-B).

#### Light chain function

If carp calcium binding protein, rabbit troponin C and the alkali light chains have similar three-dimensional structures, they may also have related functions. While the function of TNC is most clearly understood, the role of CBP remains obscure<sup>16</sup>. The presence of parvalbumins in skeletal muscles of higher ventebrates may indicate a calcium binding role directly related to muscle contraction17. There is no evidence to support any calcium binding role for the alkali light chains, and isolated light chains do not bind calcium in the concentration range  $10^{-7}$  M to  $10^{-5}$  M in the presence of 1 mM Mg2+ ions (J. Kendrick-Jones, personal communication). Thus in spite of the structural similarity, this protein may have lost an original function related to calcium binding and now possess an entirely different function, possibly connected with the ATPase activity of myosin. Experiments to demonstrate participation of these light chains in the ATPase activity of skeletal myosin18,19 have given variable results and cannot be regarded as conclusive. The reaction of the disulphide analogue of ATP with the single thiol group in the S4 corner of ALC is suggestive but not conclusive, since other thiols in the heavy chains are also labelled20.

Interest in the effects of calcium on myosin has been increased by the discovery that calcium regulation in molluscan muscles depends on a specific light chain associated with the myosin 'heads' (the EDTA light chain of scallop myosin)21. Thus mammalian muscle appears to have evolved a different form of calcium regulation, though the discovery of species with dual regulation22 has led to the speculation that vertebrate muscle might also contain a myosin regulatory system. X-ray evidence suggests a direct effect of calcium on vertebrate myosin23,24 and biochemical studies have sought to confirm this25,26. Kendrick-Jones has recently shown that of the three light chains present in rabbit myosin, one (the DTNB light chain) can substitute for the EDTA light chain of scallop myosin and restore calcium sensitivity to the desensitised myosin<sup>27</sup>. Thus this light chain acts as an inhibitor to switch 'off' the scallop actomyosin ATPase when calcium is removed. This suggests that the DTNB light chain is analogous to troponin I in the actin-linked regulatory system rather than troponin C. It is now important to establish whether the DTNB light chain has an equivalent inhibitory function in mammalian myosin and whether, in living muscle, the alkali light chains have any function related to calcium regulation.

We thank Drs H. E. Huxley and J. Kendrick-Jones for critical reading of this manuscript and helpful discussions. Note added in proof: Tufty and Kretsinger28 have kindly sent us a draft of their paper in which they have interpreted the ALC sequence in terms of three "EF hands". These three correspond to sites 1, 3 and 4 in Fig. 1c.

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## An SV40-induced initiation factor for protein synthesis concerned with the regulation of permissiveness

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An SV40-induced factor, which catalyses binding of late human adenovirus mRNA to ribosomes to form the 80S initiation complex for protein synthesis, is present in high salt extracts of ribosomes from the transformed simian cells. THE defective step of human adenovirus growth in African green monkey kidney (AGMK) cells seems to reside in the inability of ribosomes to bind with late viral mRNA for capsid proteins to form polyribosomes, since certain species of late viral mRNA are absent in polyribosomes of the infected cells

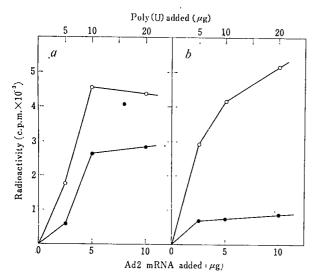


Fig. 1 Response of preincubated S30 to added RNA. The cells were washed with Earle's solution and suspended in 2 vols of TKM (10 mM Tris-HCl, pH 7.4, 10 mM KCl, 1.5 mM Mg (OAc)<sub>2</sub>) at 0° C for 10 min. The swollen cells were disrupted by about 20 strokes of a Dounce homogeniser and the tonicity was immediately restored by the addition of 1/3 vol. of tenfold concentrated medium K(1 × medium K contains 30 mM Tris-HCl, pH 7.4, 120 mM KCl, 5 mM Mg(OAc)<sub>2</sub> and 3 mM dithiothreitol (DTT)). The homogenate was centrifuged at 1,000g for 5 min to remove nuclei and the supernatant was centrifuged at 30,000g for 10 min. The supernatant (S30) was supplemented with 5 μM each of 20 amino acids, 5 mM ATP, 0.5 mM GTP, 6 mM phosphoenolpyruvate (PEP), 10 μg ml<sup>-1</sup> of pyruvate kinase and incubated at 37° C for 50 min. The incubated S30 was centrifuged at 10,000g for 5 min and the supernatant was dialysed against medium K. The reaction mixture (0.15 ml) for amino acid incorporation contained 50 μl of preincubated S30, 1 mM ATP, 0.1 mM GTP, 10 mM PEP, 0.2 mg ml<sup>-1</sup> of pyruvate kinase, 20 μM of 19 naturally occurring L-amino acids (minus phenylalanine or leucine) and 1μCi ml<sup>-1</sup> of <sup>3</sup>H-phenylalanine (10 Ci mmol<sup>-1</sup>) or <sup>3</sup>H-leucine (32 Ci mmol<sup>-1</sup>) in the presence of either poly(U) (○) or Ad2 mRNA (●). The mixture was incubated at 37° C for 1 h and the hot trichloroacetic acid (TCA)-insoluble count was measured. a, T22 cell S30; b, C14 cell S30.

although they are present in the cytoplasm<sup>1</sup>. This defect, however, can be complemented either by coinfection or transformation of the cells with SV40, indicating that a factor(s) is induced by SV40 which might enable the ribosomes to bind with this species of late adenovirus mRNA.

Initiation of protein synthesis in *Escherichia coli* requires three factors (F1, F2 and F3)<sup>2-5</sup> that associate with ribosomes, and similar factors (M1, M2 and M3)<sup>6-8</sup> have been found in mammalian cells. Of these, F3 (M3) is concerned with mRNA selection by ribosomes and, therefore, has the regulatory function of deciding the species of mRNA to be translated. The function of this factor can easily be modified as has been demonstrated in *E. coli* after infection with phage T4 (ref. 9), T7 (refs 10 and 11) or MS2 (ref. 12).

Here we show that a factor(s) is present in the high salt extract of ribosomes from SV40-transformed AGMK cells which are permissive for human adenoviruses, but not in ribosomes from untransformed AGMK cells which are non-permissive. The factor enables ribosomes to bind with late adenovirus mRNA and to form the 80S initiation complex for protein synthesis. We discuss a mechanism of induction of this factor by SV40 in relation to the mechanism of cell transformation by oncogenic viruses.

#### Response of preincubated S30 to added RNA

Differential response to RNA of ribosomes prepared from SV40-transformed AGMK cells, clone T22 (ref. 13), and untransformed AGMK cells, clone C14 (ref. 13), was compared by protein synthesis with preincubated S30 in response to either late adenovirus type 2 (Ad2) mRNA or poly(U). Late Ad2

mRNA was prepared as poly(A)-containing RNA from polyribosomes of the infected KB cells as reported previously<sup>1</sup>. The RNA was sedimented in an SDS-sucrose gradient and the 24S region was collected because the size of mRNA for hexon, a major component in adenovirus capsid proteins, was estimated as 24S on the basis of the molecular weight of hexon, 130,000 (ref. 14). A certain species of mRNA of this size is apparently absent from polyribosomes of the adenovirusinfected C14 cells but present in the infected T22 cells<sup>1</sup>. As shown in Fig. 1, C14 cell S30 translates late Ad2 mRNA very poorly by comparison with T22 cell S30, whereas both translate poly(U) with almost the same efficiency. A slight incorporation of amino acids by C14 cell S30 in response to late Ad2 mRNA is probably caused by contamination of cellular mRNA, although poly(A)-containing RNA separated on poly(U)-Sepharose consists predominantly of Ad2 mRNA as described previously1. The gel electrophoretic analysis of the products (Fig. 2) showed that hexon was synthesised with T22 cell S30 in response to late Ad2 mRNA but not with C14 cell S30. These results indicate that a factor(s) responsible for translation of certain species of late Ad2 mRNA is present in SV40-transformed AGMK cells but not in untransformed AGMK cells.

#### Formation of 80S initiation complex

The presence of an initiation factor which catalyses binding of late Ad2 mRNA to ribosomes in the high salt extract of ribosomes (ribosomal wash) from SV40-transformed AGMK cells was demonstrated by formation of the 80S initiation

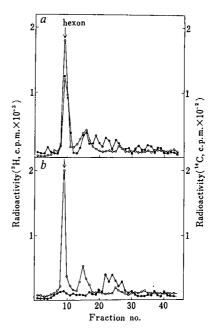


Fig. 2 Gel electrophoretic analysis of product synthesised with S30. Protein synthesis with S30 in response to late Ad2 mRNA was carried out as described in Fig. 1, except that ³H-leucine and 19 cold amino acids were replaced by ¹⁴C-amino acid mixture. After the reaction, pH was adjusted to 11.0 by the addition of 0.1 N NaOH and the mixture was incubated at 37° C for 15 min. The mixture was dialysed against 0.1 M sodium phosphate (pH 7.4) containing 0.1% SDS and 0.01% 2-mercaptoethanol overnight. The products were concentrated by evaporation at 37° C in dialysis tubing and Ad2 virions labelled with ³H-amino acids mixture were added. After heating at 100° C for 1 min, the products were subjected to electrophoresis. Electrophoresis was carried out on 7.5% polyacrylamide gel (10×0.6 cm) at 80 V (15 mA per gel) for 4 h. The buffer used was 0.05 M sodium phosphate (pH 7.2) containing 0.1% sodium dodecyl sulphate (SDS). Gels were then sliced and the slices were counted in 5 ml of Triton X-toluene scintillator after dissolution in 0.5 ml of 50% H<sub>2</sub>O<sub>2</sub> at 80° C overnight. a, T22 S30; b, C14 S30. ♠, ¹⁴C; ○, ³H.

complex for protein synthesis. Ribosomes washed with 0.5 M KCl were prepared from preincubated C14 cells S30. High salt extract of ribosomes was prepared from cells either permissive or non-permissive to adenoviruses. Met-tRNA<sub>f</sub> was prepared by charging methionine to tRNA from T22 cells with *E. coli* S100.

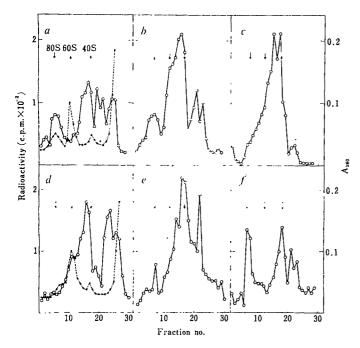


Fig. 3 Formation of the 80S initiation complex with Ad2 mRNA. Ribosomes were prepared from the preincubated C14 cell S30. The S30 was treated with sodium deoxycholate (0.8%) and centrifuged at 16,000g overnight through a cushion of 60% sucrose in medium K at 4° C. The pellet was suspended in 1 ml of 50 mM triethanolamine-HCl (TEA), pH 7.6, containing 0.5 M KCl, 1.5 mM Mg(OAC)2 and 1 mM puromycin²2 and incubated at 0° C for 10 min followed by incubation at 37° C for 10 min. It was centrifuged through a 10-30% (w/w) sucrose gradient in 50 mM TEA, pH 7.6, containing 0.5 M KCl and 5 mM Mg(OAC)2 at 23,000 r.p.m. for 16 h at 4° C in a Spinco SW 25.1 rotor. The 40S and 60S ribosomal subunits, collected separately from the gradient, were stored at 0° C in medium K containing 20% glycerol after centrifugation at 16,000g for 5 h. Ribosomal wash fraction was prepared from crude ribosomes obtained from the preincubated S30. Ribosomes were gently stirred for 1 h at 0° C in 0.25 M sucrose containing 50 mM Tris-HCl, pH 7.4, 10 mM MgCl2, 1 M KCl, 2 mM DTT, 10 mM 2-mercaptoethanol and centrifuged at 200,000g for 4 h at 4° C through a cushion of 0.5 M sucrose in the same solution. The top supernatant was dialysed overnight against 80% ammonium sulphate in 50 mM Tris-HCl, pH 7.4, containing 50 mM KCl, 0.1 mM EDTA, 10 mM 2-mercaptoethanol and 5% glycerol dialysed against the same buffer for 4 h at 0° C and stored at —80° C. Transfer RNA was prepared from T22 cells and amino-acylated by S100 extract of E. coli. Formation of the 80S initiation complex was carried out in four successive incubation steps according to Levin et al. The components added in each reaction step were dissolved in 40 mM Tris-HCl, pH 7.4, containing 1 mM DTT and 0.4 mM GTP. Each reaction was carried out at 37° C for 5 min. Step 1; (0.1 ml) 30 pmol of Met-tRNA, 75µg of ribosomal wash proteins, 0.1 M KCl. Step 2: (0.1 ml) 0.25 A<sub>280</sub> units of 40S subunits, 80 mM KCl and 5 mM MgCl2. Step 3: (0.05 ml) 9.1 Helabelled late Ad2 mRNA (about 5,000 c.p.m.), 65 mM KCl an

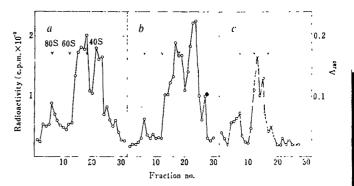


Fig. 4 Formation of the 80S initiation complex with SV40 mRNA. Formation of the initiation complex was carried out as described in Fig. 3. Ribosomal wash proteins (75 µg) used were prepared from: a, T22 cells; b, C14 cells; c, GC7 cells. SV40 mRNA was prepared as poly(A)-containing RNA from polyribosomes of the infected GC7 cells at 48 h after infection as previously described.

The binding of mRNA to ribosomes was carried out with either 3H-labelled late Ad2 or SV40 mRNA in four successive incubation steps according to Levin et al.15 (Figs 3 and 4). Late Ad2 mRNA prepared from KB cell polyribosomes forms the 80S initiation complex with T22 and KB cell ribosomal wash (Fig. 3a and b) but not with GC7 and C14 cell ribosomal wash (Fig. 3c and d); GC7 cells are also a line of AGMK cells, non-permissive to adenoviruses. A small shoulder observed at 80S in Fig. 3c and d may indicate the presence of a small amount of cellular mRNA in Ad2 mRNA preparation. The ribosomal wash prepared from a permissive monkey kidney cell line, Vero cells, formed a smaller amount of the 80S complex (Fig. 3e), which may reflect the lower susceptibility of Vero cells to adenovirus infection than KB and T22 cells. Late Ad2 mRNA prepared from the cytoplasm of infected C14 cells formed the complex with the T22 cell ribosomal wash with the same efficiency as late Ad2 mRNA prepared from polyribosomes of the infected KB cells (Fig. 3f) indicating the functional integrity in formation of the 80S complex of late

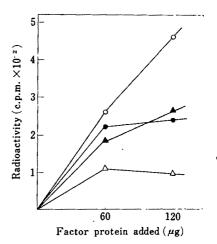


Fig. 5 Formation of methionyl-puromycin. The reaction mixture (0.3 ml) for formation of the 80S initiation complex containing <sup>3</sup>H-met-tRNA (about 50 pmol, 20,000 c.p.m.) and either 8 μg of late Ad2 mRNA or 20 μg of poly(AUG) (Sigma) was incubated at 37° C with increasing amounts of ribosomal wash proteins as described in Fig. 3. Puromycin was then added to a final concentration of 1 mM (final volume 0.5 ml) and incubation was continued at 37° C for 30 min. The reaction was terminated by the addition of 0.5 ml of 0.2 M sodium phosphate buffer (pH 8.1) and <sup>3</sup>H methionyl-puromycin was extracted<sup>23</sup> with 1.5 ml of ethyl acetate saturated with the same buffer. An aliquot (1 ml) of the ethyl acetate layer was counted in 10 ml of Bray's scintillator. O, Ad2 mRNA, T22 cell ribosomal wash; Δ, Ad2 mRNA, C14 cell ribosomal wash; Φ, poly(AUG), C14 cell ribosomal wash.

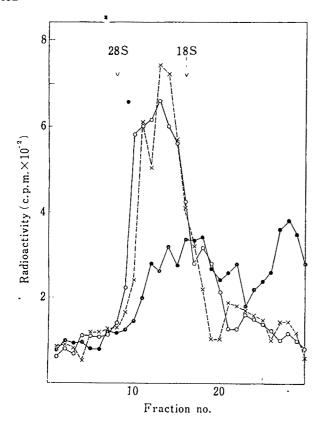


Fig. 6 Size distribution of late Ad2 mRNA after binding reaction. The reaction for formation of the 80S initiation complex with 3H-labelled late Ad2 mRNA was carried out as described in Fig. 3 with ribosomal wash proteins from either T22 cells (O) or C14 cells (•). The reaction was terminated by the addition of 0.5% SDS and the mixture was centrifuged in a 15-30% (w/w) SDS-sucrose gradient at 24,000 r.p.m. for 18 h at 28° C in a Spinco SW 25.1 rotor. Each fraction was counted after TCA precipitation. ×, 3H-labelled late Ad2 mRNA was centrifuged without reaction.

Ad2 mRNA transcribed in non-permissive monkey cells. The formation of the complex depended on Met-tRNA<sub>f</sub>. The inability of C14 and GC7 cell ribosomal wash to bind late Ad2 mRNA to ribosomes does not result from an inactivation of factor proteins. The 80S initiation complex with SV40 mRNA was formed with ribosomal washes from C14 and GC7 cells which are permissive to SV40 (Fig. 4). The ribosomal washes from T22 and C14 cells facilitated binding of poly(U) to ribosomes at low concentrations of Mg2+ with the same efficiency, indicating that both ribosomal washes contain initiation factors M1 and M2. The mRNA-protein complex slightly larger than 40S was formed with the ribosomal wash from both permissive and non-permissive cells (Figs 3 and 4). It is not yet clear whether this complex is the 40S initiation complex or the ribonucleoprotein particle formed between late Ad2 mRNA and proteins in the ribosomal wash.

#### Functional integrity of 80S complex

To demonstrate that the 80S complex formed with late Ad2 mRNA is active in the initiation of protein synthesis, the formation of methionyl-puromycin (met-puro) in the reaction mixture with the ribosomal wash from both T22 cells or C14 cells was examined. Formation of met-puro increased linearly with increasing amounts of T22 cell ribosomal wash (Fig. 5), whereas with C14 cell ribosomal wash it reached a plateau after a small increase. This difference was not observed when late Ad2 mRNA was replaced with poly(AUG). Addition of 120 μg each of C14 and T22 cell ribosomal wash to the binding mixture did not alter the amount of met-puro formed by T22 cell ribosomal wash alone, indicating that an inhibitor of the formation of either the intiation complex or dipeptide is not present in C14 cell ribosomal wash. The stability of late Ad2 mRNA during the binding reaction was studied by examining the alteration in its size. The size distribution of late Ad2 mRNA in an SDS-sucrose gradient after the binding reaction with T22 cell ribosomal wash was the same as that of nonincubated late Ad2 mRNA (Fig. 6), whereas the binding reaction with C14 cell ribosomal wash resulted in the degradation of late Ad2 mRNA. RNase activity in the ribosomal wash was low and not significantly different for C14 and T22 cells. These results indicate that formation of the functional complex with ribosomes protects mRNA from degradation and that the factor present in the ribosomal wash of T22 cells is specific for late Ad2 mRNA.

#### Genesis and biological significance

These results provide the first evidence that the permissiveness and non-permissiveness of the cells to virus infection is regulated at the initiation of protein synthesis. They also offer a good system for characterising the nature of the initiation factor M3. Appearance of a new initiation factor responsible for late Ad2 mRNA translation in SV40-transformed simian cells may be the result of either modification of a pre-existing M3 factor or induction of a new M3 factor by the SV40 gene function. The SV40 helper function for growth of human adenoviruses in monkey cells is one of the virus early functions, since an early mutant of SV40, tsA7 (ref. 16), defective in virus DNA replication, expresses this function at the non-permissive temperature. An adeno 2-SV40 hybrid virus, Ad2+ND1 (refs 17 and 18) in which only 18% of the SV40 DNA is integrated into Ad2 DNA, expresses the SV40 function to induce U antigen and to support adenovirus growth in simian cells. The factor itself may not be coded by the SV40 genome, however, as the early region estimated by hybridisation competition is too small<sup>19,20</sup> to code various proteins and the SV40 induced-U antigen is localised in the nuclei of the infected cells. The factor may be synthesised as a result of derepression of the host cell genome by some unknown SV40 early gene product. Our findings indicate that the efficiency of the helper function in supporting synthesis of Ad2 virion antigen in simian cells previously infected with SV40 (24 h before infection of adenoviruses) depends on the multiplicity of SV40 infection and correlates with the efficiency of induction of cellular mRNA synthesis. The alteration of ribosomes in the SV40-infected simian cells so as to respond to late Ad2 mRNA to synthesise hexon is also multiplicity-dependent. These results support the assumption that the derepression of the cellular function is involved in the helper function. Derepression of the cellular function by SV40 may cause the pleiotropic changes in the cells which would result in the alteration of various cellular systems, such as the cell surface structure21 and the protein-synthesising system, causing the cell to become malignant.

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# **Echinomycin:** a bifunctional intercalating antibiotic

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The interaction between echinomycin and circular DNA or sonicated rod-like DNA fragments shows that this antibiotic binds by a bifunctional mode of intercalation. Binding parameters for a variety of natural and synthetic DNAs vary widely, indicating that echinomycin interacts selectively with specific nucleotide sequences.

THE significance of twofold symmetry in the structures of small molecules which bind to DNA, itself characterised by a dyad axis of symmetry perpendicular to the helix axis, was emphasised by Sobell et al.1 in their model for actinomycin-DNA binding derived from a crystallographic study of a 1:2 actinomycindeoxyguanosine complex. Subsequent work has shown the importance of symmetry-related elements in the recognition of DNA sequences by repressors<sup>2,3</sup>, restriction enzymes<sup>4</sup> and possibly transcriptional factors4.5, suggesting that principles of symmetry may prove to be a general feature of recognition processes involving DNA.

We were struck by the apparently perfect symmetry in the structure of the antibiotic echinomycin<sup>6</sup> proposed by Keller-Schierlein et al.7. Echinomycin has long been known to bind to DNA<sup>8,9</sup> but the nature of its interaction has remained obscure. It is an extremely potent inhibitor of RNA synthesis<sup>8-10</sup>, some

four to five times more potent than actinomycin D in vivo10, and there is every indication that its antitumour and other biological activities result from its binding to DNA<sup>11</sup>. During the course of our work, proton magnetic resonance studies<sup>32</sup> suggested that echinomycin contained four more hydrogen atoms than expected from the published structure?. While the revised structure (Fig. 1) retains a twofold axis of rotational symmetry for the cyclic octapeptide dilactone and its attached quinoxaline-2-carboxylic acid chromophores, the sulphur-containing cross-bridge (replacing the dithian ring originally proposed<sup>7</sup>) introduces an element of asymmetry. In respect of this pseudo-symmetrical appearance, as well as the sequence of amino acid residues in the two halves, there are obvious parallels with the structure of actinomycin1.

#### Specificity for double-helical DNA

Echinomycin is much less soluble in purely aqueous systems than actinomycin, which necessitated the development of a novel solvent-partition method to investigate its binding to nucleic acids. Representative results, full details of which will be published elsewhere, are shown in Fig. 2. These curves reveal that the binding is completely specific for DNA, not RNA, that single-stranded or heat-denatured DNAs bind less antibiotic than intact double-helical DNA, and that complete disruption

1 Structure of echinomycin. Abbreviations: MeVal, methylvaline; Ser, serine; Ala, alanine; Cys, cysteine; DiMe-L- Cys, N,S-dimethyl-L-cysteine. The formula is a revision of that published previously7, based on new results from mass spectrometry and nuclear magnetic resonance spectroscopy32.

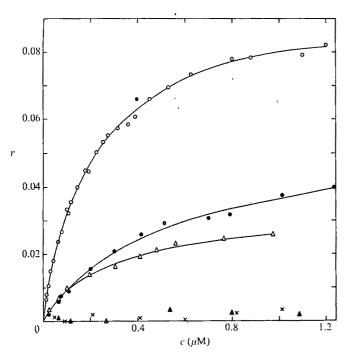


Fig. 2 Interaction between echinomycin and nucleic acids. The buffer (SHE) contained 2 mM HEPES, 10 μM EDTA and 9.4 mM NaCl (pH 7.0 at 20° C; total ionic strength 0.01). Portions (3 ml) of nucleic acid solutions (182 μM with respect to nucleotides) were shaken together with 4 ml of echinomycin dissolved in iso-amyl acetate for 2 h at 20.0° C to establish equilibrium. The phases were then separated by centrifugation at 2,000 r.p.m. for 30 min in an MSE Super Minor centrifuge. The free antibiotic concentration in the aqueous phase (c) was determined from the absorbance of the organic phase at 315 nm measured in a 40 mm light path semimicro cuvette, based on a molar extinction coefficient of 1.15 × 10⁴ for echinomycin in iso-amyl acetate and its partition coefficient between this solvent and SHE buffer (8.93 ± 0.32 × 10<sup>-3</sup> at 20° C). The total antibiotic concentration in the aqueous phase was determined by measuring the absorbance at 325 nm after dissociating the complex by addition of an equal volume of dimethyl sulphoxide (DMSO). In 50% (v/v) DMSO-buffer mixtures the molar extinction coefficient was determined to be 1.24 × 10⁴ at 325 nm irrespective of the nucleic acid concentration. The concentration of bound echinomycin was obtained by difference and expressed in terms of r, mol antibiotic bound per mol of nucleotides. O, Native calf thymus DNA sheared to produce fragments of approximately 18S free from single-stranded ends by the method of Pyeritz et al.³0; ♠, single-stranded circular DNA from bacteriophage fd; △, calf thymus DNA denatured by rapid cooling; ♠, calf thymus DNA denatured by rapid cooling; ♠, calf thymus DNA denatured in the presence of 1% formal-dehyde; ×, total ribosomal RNA from E. coli B.

of secondary structure by denaturation of DNA in the presence of formaldehyde abolishes the binding altogether. A Scatchard plot of the data for calf thymus DNA yielded a straight line up to an revalue (antibiotic molecules bound per nucleotide) of about 0.06; the slope and intercept of this line gave an apparent association constant  $K_{\rm ap}$  of  $6.0\pm0.3\times10^6$  M<sup>-1</sup> and the number of sites per nucleotide (n) as 0.087, equivalent to one binding site per six base pairs in this DNA. Values of  $K_{ap}$  and n for the heat-denatured DNA were  $4.0\pm0.4\times10^8$  M<sup>-1</sup> and 0.032, respectively; for fd DNA they were 2.2±0.2×10<sup>6</sup> M<sup>-1</sup> and 0.053. These parameters, two to three times lower than those for the native double-helical DNA, undoubtedly reflect the limited interaction between echinomycin and 'hairpin' helical segments present in the single-stranded DNAs; witness the lack of interaction after formaldehyde treatment. These results corroborate the qualitative conclusions of earlier workers8,9 and compare well with parameters reported for the binding of actinomycin to DNA<sup>12,13</sup>.

#### Bifunctional intercalation

The quinoxaline chromophores of echinomycin are similar in size to the quinoline chromophore of chloroquine, which is

known to bind to DNA by intercalation<sup>11,15</sup>. Their symmetrical disposition with respect to the octapeptide ring, together with the apparent structural and functional resemblance between echinomycin and actinomycin, suggested that both chromophores might be capable of intercalation, in a symmetry-related fashion, at the same time. A double-intercalation event would be expected to result in twice the unwinding of the helix caused by binding of a monofunctional intercalating agent<sup>15</sup>, twice the extension along the helix axis<sup>16</sup>, and markedly enhanced binding to negatively superhelical circular DNA at low values of r (ref. 17). These predictions have been verified.

Figure 3 compares the unwinding of the helix associated with binding of echinomycin to that caused by ethidium, an established (monofunctional) intercalating drug<sup>15.18.19</sup>. Echinomycin removes and reverses the supercoiling of circular PM2 DNA in decisive fashion, with an equivalence binding ratio<sup>15</sup> of 0.028 ± 0.004 antibiotic molecules bound per nucleotide. Under the same conditions equivalence occurs with 0.051 ±0.007 ethidium molecules bound per nucleotide. Thus the helix-unwinding angle of echinomycin is 1.82 ±0.30 times that of ethidium, or 21.9° per bound antibiotic molecule if the estimate of 12° unwinding for ethidium<sup>18</sup> is accepted<sup>15.19</sup>. This is the first time a drug has been found which unwinds the DNA helix by an angle larger than does ethidium, the actual value being almost double.

Figure 4a shows the extension of the helix caused by binding of echinomycin,  $1.87\pm0.05$  times the theoretical extension of 3.35 Å required to accommodate a single aromatic ring system, corresponding to a lengthening of about 6.3 Å per bound antibiotic molecule. Comparable experiments with proflavine and ethidium were reported to yield a value of  $2.7\pm0.2$  Å (refs 16 and 20). Again the value for echinomycin is twice as high.

Figure 4b compares binding isotherms for the interaction between echinomycin and closed or nicked circular PM2 DNA. They cross at an r value of about 0.027, confirming the equivalence binding ratio determined in the sedimentation experiments (Fig. 3a) and corresponding to an unwinding angle 1.89 times

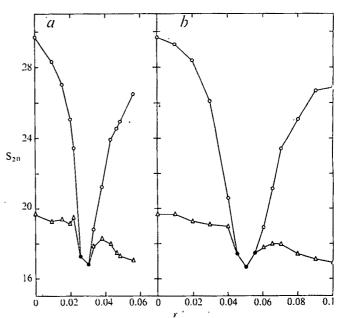


Fig. 3 Effects of (a) echinomycin and (b) ethidium bromide on the sedimentation coefficient of PM2 DNA in SHE buffer. The DNA preparations of contained 80-90% closed circular duplex molecules. Ο, S<sub>20</sub> of closed circles; Δ, that of nicked circles; Φ, weight-average S<sub>20</sub> when the relaxed closed circular molecules cosedimented with the nicked circles. Ultracentrifugation experiments and spectrophotometric measurements of ethidium-DNA binding were as previously described is. Echinomycin-DNA complexes were formed by adding small volumes of antibiotic dissolved in acetone to the DNA in SHE buffer; the acetone was then removed under reduced pressure. The binding ratio (r) was determined from a curve constructed as in the legend to Fig. 2. Sedimentation coefficients are uncorrected values determined directly at 20° C.

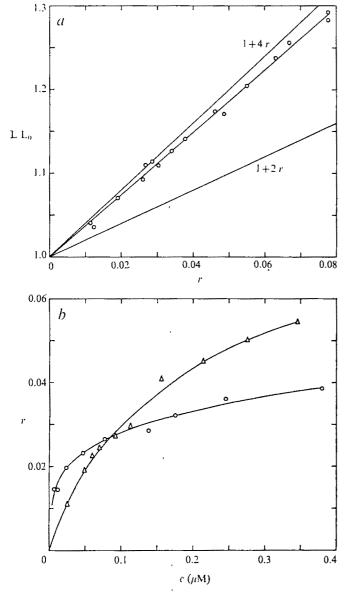


Fig. 4 a, Relative length increase  $L/L_0$  of echinomycin-DNA complexes as a function of the binding ratio.  $L/L_0$  was calculated from viscosity measurements on sonicated rod-like DNA fragments in SHE buffer at  $20^{\circ}$  C using a capillary viscometer as described by Cohen and Eisenberg<sup>16</sup>. The DNA fragments were prepared by sonicating calf thymus DNA to a molecular weight of  $5.4 \times 10^{\circ}$  in an MSE 150 W ultrasonic disintegrator; their intrinsic viscosity in the absence of antibiotic was 3.9 dl g<sup>-1</sup>. Complexes of known binding ratio (r) were prepared as in Fig. 2. The line fitted to the points is a least-squares line, constrained to pass through the origin, of slope  $1+3.73\,r$ . Also shown are theoretical lines corresponding to the lengthening expected for ideal monofunctional intercalation  $(1+2\,r)$  and ideal bifunctional intercalation  $(1+4\,r)$ . b, Binding of echinomycin to closed  $(\bigcirc)$  and nicked  $(\triangle)$  circular PM2 DNA in SHE buffer, determined as described in Fig. 2. The sample of nicked circles was generated by repeated freezing and thawing.

greater than that of ethidium. More important, the enhanced binding to closed circles at ratios below the crossover, and restricted binding above it, are clearly seen. The differential binding to the two forms of DNA at low r is considerably more marked than that reported for ethidium<sup>21</sup>, as expected if the ratio of equilibrium constants for closed circles compared to nicked circles  $(K_I/K_{II})$  is an exponential function of the unwinding angle<sup>17</sup>. Equations (4) and (7) of Davidson<sup>17</sup> predict a value of  $K_I/K_{II} = 5.8$  for echinomycin binding to a typical closed circular DNA at  $r \to 0$ , based on an unwinding angle of 22°. One consequence of this is that any biological property resulting solely from preferential interaction with negatively superhelical

circular DNA would be amplified some 2.23 times with echinomycin  $(K_{\rm I}/K_{\rm II}=5.8)$  in comparison with ethidium  $(K_{\rm I}/K_{\rm II}=2.6)^{17}$ . Furthermore, detailed measurements of the interaction between echinomycin and a range of natural and artificially-closed circular DNAs should enable more accurate estimation of such parameters as the superhelix free energy<sup>21</sup>, in addition to providing a more sensitive probe for the biological relevance of circularity in DNA.

#### Nature of the binding site

The unwinding angle and extension of the helix per bound echinomycin molecule, being double the values observed with classical intercalating drugs, lend strong support to the argument that each chromophore participates in an intercalation process symmetrically related to the binding site of its partner. The two intercalation sites are presumably distinct, because it is practically inconceivable that both chromophores might be accommodated in the potential space between one set of adjacent base-pairs. The symmetry argument would therefore predict that whatever constraints apply to the intercalation site for one chromophore should apply in antiparallel fashion to the site for the other. In the extreme case where each chromophore demanded one specific site out of the ten distinguishable types of potential space<sup>19</sup>, the specificity of binding of the antibiotic as a whole would be phenomenal: one in 40 or one in 100 possible locations along the length of a random sequence would be acceptable, depending on the number of base pairs (1 or > 1)between the intercalated chromophores.

Echinomycin lacks that extreme specificity. Nevertheless, there is some form of subtle specificity in its interaction with DNA. Binding parameters determined for a range of natural DNAs having G+C contents from 29 to 72% yielded values of  $K_{\rm ap}$  from  $4.5\times10^6$  M<sup>-1</sup> to  $4.0\times10^7$  M<sup>-1</sup> with a tendency for the DNAs richer in G+C to bind the antibiotic more tightly. However, two DNAs of identical base composition (*Escherichia* 

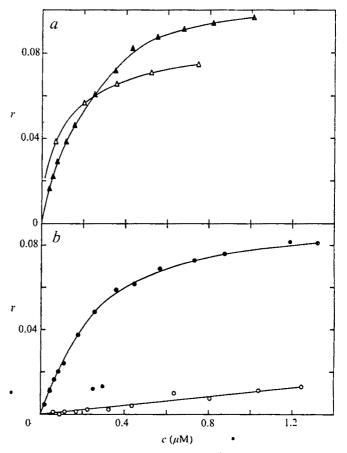


Fig. 5 Interaction between echinomycin and poly(dA) poly(dT) ( $\bigcirc$ ), poly d(A-T); ( $\bigcirc$ ), poly(dG) poly(dC); ( $\triangle$ ) and poly d(G-C); ( $\triangle$ ) in SHE buffer at 20° C, measured as in Fig. 2.

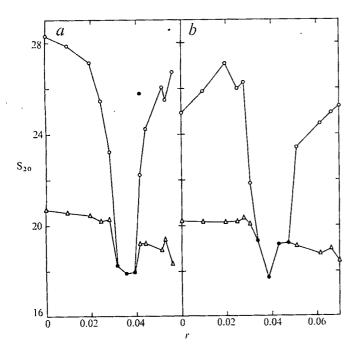


Fig. 6 Effect of echinomycin on the sedimentation coefficient of PM2 DNA at ionic strength 0.1 (a) and 0.5 (b). Symbols and methods as described in the legend to Fig. 3. The buffers were SHE supplemented with NaCl to the required ionic strength. At ionic strength 0.1 equivalence occurs with  $0.036 \pm 0.006$  echinomycin molecules bound per nucleotide; at ionic strength 0.5 the value rises to  $0.041 \pm 0.007$ . In the same buffers equivalence binding ratios for ethidium are  $0.055 \pm 0.005$  and  $0.048 \pm 0.009$ , respectively 25.

coli and Salmonella typhimurium; both 50% G+C) yielded significantly different association constants. Numbers of sites derived from Scatchard plots fell within the range 0.054–0.087 sites per nucleotide, but no correlation with the base composition was apparent.

Striking differences were found when the binding to synthetic DNA polymers was investigated (Fig. 5). Three polymers bound the antibiotic well, albeit with widely different binding constants that is, poly(dG)·poly(dC), poly d(G-C) and poly d(A-T). By contrast, poly(dA) poly(dT) showed barely detectable binding (Fig. 5); neither did poly(dI) poly(dC), poly d(I-C) and poly(rA)·poly(rU), notwithstanding their regular helical secondary structure. The data for the three polymers which showed strong interaction were re-plotted according to the statistical mechanical treatment of Zasedatelev et al.22, from which the number of base pairs occluded per bound echinomycin molecule was calculated to be three for poly d(A-T) and poly d(G-C) but nearer four for poly(dG) poly(dC). The interactions with synthetic polydeoxyribonucleotides will be discussed fully elsewhere, but it should be noted that the capacity of a given polymer to bind echinomycin is not simply correlated with its precise helical form23, nor with the extent to which its helical structure resembles the B-form of natural DNAs. Since coliphage T2 DNA, whose major groove is largely filled by glucosyl substituents, bound the antibiotic well ( $K_{ap} = 1.5 \pm 0.1$  $\times 10^7$  M<sup>-1</sup>; n = 0.054) it is likely that the binding site for the non-intercalated peptide portion of echinomycin lies in the minor groove, as is believed to be true for actinomycin<sup>1,13</sup>. If so, the presence and/or orientation of the 2-amino group of guanine is likely to have important influence on the binding reaction, as is known to be the case with actinomycin<sup>1,13,24</sup> This is clearly implied by the different binding properties of polymers apparent in Fig. 5.

#### Physical characteristics of the binding reaction

The equilibrium constant for binding to native calf thymus DNA at ionic strength 0.01 showed no detectable variation

with temperature between 20 and 40°C. Thus  $\Delta H$  must be close to zero and the binding reaction is driven by a large positive entropy change,  $\div 31$  e.u. per mol of echinomycin bound. These parameters are in exact agreement with those reported for actinomycin binding under almost identical conditions<sup>12</sup>. Unlike actinomycin, however, the solubility of echinomycin in aqueous systems is practically independent of ionic strength and temperature and remains about 5  $\mu$ M. This limited solubility corresponds to a negative entropy change of approximately 25 e.u., suggesting that the major driving force for the binding reaction is the transfer of echinomycin from an aqueous phase into a hydrophobic environment.

When the salt concentration is raised the character of the binding reaction changes. At ionic strength 0.1 the value of  $\Delta S$ falls drastically to near zero and the  $\Delta H$  of binding becomes  $-8.5\pm2.6$  kcalorie mol<sup>-1</sup>. At the same time the equivalence binding ratio for PM2 DNA increases relative to that of ethidium, yielding an apparent unwinding angle of  $18.4\pm3.0^{\circ}$ ,  $1.54\pm0.25$  times that of ethidium (Fig. 6a). In the same solvent, viscometric measurements like those presented in Fig. 4a reveal an extension of the helix corresponding to 1+3.0 r, that is,  $1.50\pm0.05$  times the theoretical extension for a single intercalation event. On raising the ionic strength to 0.5 the trend is continued (Fig. 6b). The unwinding angle becomes  $1.18\pm0.19$ times that of ethidium, and the helix extension falls to  $1.16\pm0.03$ times the value for ideal monofunctional intercalation. The agreement between these totally independent assays of the effect of echinomycin binding on the DNA helix leaves little room for doubt that at ionic strengths 0.01, 0.1 and 0.5 the binding occurs by bifunctional, sesquifunctional, and almost monofunctional intercalation respectively.

#### Structural requirements

The cross-bridged symmetrical octapeptide ring of echinomycin is not merely a convenient handle on which two potentially intercalative chromophores are mounted. In order to investigate the possible role of the chromophores in isolation we studied the interaction of quinoxaline-2-carboxamide (a gift from Dr A. Dell) with DNA. This compound (O2C) produced no detectable effect on the sedimentation of closed circular PM2 DNA at Q2C/nucleotide ratios up to 1:1 in buffer of ionic strength 0.01. Moreover, binding was undetectable by perturbation of ultraviolet absorption spectra or equilibrium dialysis under conditions<sup>25</sup> where interaction characterised by a binding constant as low as 2×103 M<sup>-1</sup> would have been measurable, a factor of 3,000 lower than the binding constant for echinomycin itself. Clearly the tight binding of echinomycin to DNA cannot derive solely from intercalation of the chromophores but must involve interactions with functional substituents elsewhere in the intact molecule, that is, on the peptide ring. Nevertheless, although the chromophores alone do not appear to be able to bind to DNA they undoubtedly play a vital part in dictating the geometry of the antibiotic-DNA complex on which such sequence-specificity as exists must depend. Their intercalation causes the helix to unwind and it is in this distorted configuration that interactions between the peptide portion of the antibiotic and substituents in the DNA must be optimised. Such interactions with the undistorted helix may be unfavourable or indeed impossible.

The role of the bridge across the two antiparallel halves of the peptide ring may be more critical. Echinomycin belongs to a family of chemically related metabolites produced by streptomycetes, known generically as quinoxaline antibiotics<sup>11,26</sup>. This family includes the triostins, which are also antibacterial and antitumour agents<sup>11</sup> capable of powerfully inhibiting RNA synthesis<sup>10</sup>. Triostin A is structurally homologous to echinomycin except that the cross-bridge is formed by N,N'-dimethyl-cystine<sup>26</sup>. Triostin C differs further in that the N-methyl-valine residues are replaced by N,γ-dimethyl-L-alloisoleucine<sup>26</sup>. Both triostins A and C were found to remove and reverse the supercoiling of PM2 DNA but the ratios required to reach equivalence, while still lower than the equivalence ratio for

ethidium, were higher than the equivalence ratio for echinomy-

#### **Implications**

Although drugs which bind to DNA are of paramount importance in several areas of chemotherapy27, not least the treatment of cancer, their capacity to discriminate between DNAs from different sources is minimal if not actually nonexistent19. Few exhibit anything approaching sequence-specificity, the preference of actinomycin for binding to GpC sequences being a possible exception<sup>1,24</sup>. Echinomycin appears to be little better, but at least its capacity for bifunctional reaction heralds the possibility of gaining more genuine sequence specificity. Absolute specificity might be achieved through direct interaction between functional groupings on the basepairs and the drug molecule, or indirectly via recognition of a local conformational peculiarity of the DNA associated with a particular nucleotide sequence23.28. There is evidence that either or both of these phenomena are features of echinomycin-DNA interaction, and we are currently exploring the details in order to develop a precise molecular model for the binding process. Once the exact intermolecular contacts are established, and the importance of the symmetrical structure of the antibiotic is clarified, the way will be open to devise means of enhancing the selectivity of binding. Naturally-occurring analogues with various amino acid substitutions exist, and some analogues with modified chromophores have been synthesised<sup>11</sup>.

A detailed molecular model should also throw light on two outstanding problems concerning the intercalation reaction itself, that is, the origins of the neighbour-exclusion phenomenon (if it exists)19,22,29 and the absolute magnitude of helix-unwinding angles which are currently referred to an assumed value of 12° for ethidium<sup>15,18,21,25</sup>. It may also add to our understanding of how macromolecules such as repressors, transcriptional factors, restriction enzyme's and polymerases recognise specific loci in DNA, and the role of symmetry relationships in such recognition.

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# New observations of the angular diameterredshift relation for radio sources

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Measurements of the angular sizes of radio source components in the range 0.1"-1.0", using the technique of interplanetary scintillation, reveal an absence of small-diameter sources at the largest redshifts. The cosmological implications of this result are discussed.

It is well known that the angle subtended by a 'standard rigid rod' varies with redshift in a manner which depends upon the assumed cosmological model. In classical world models this angle exhibits a minimum near z=1 which contrasts with the linear decrease of angle obtained for Euclidean geometry. Attempts to use the observed angular diameters of optical galaxies and radio sources to discriminate between different cosmologies have run into various difficulties. The subtleties of applying the test to galaxies have been described by Sandage1 and although progress has been made towards solving some of the problems2 the observations do not yet extend to large enough redshifts for a decisive result to be obtained. Radio

sources are characterised by a large spread of physical sizes which has made them unsatisfactory as rigid rods. When the largest angular sizes of radio sources having steep spectra are plotted against redshift an approximately linear decrease is found to a redshift  $z\sim2$ . Miley has suggested that this apparent Euclidean behaviour might be due to cosmological evolution such that the physical size of a source is smaller at earlier epochs.

Here we use new observations made at a higher angular resolution by the method of interplanetary scintillation.

#### Scintillating sources as standard rods

A radio source will not scintillate strongly unless it contains components which, individually, subtend an angle of less than 2" in any direction and our study is therefore confined to compact features within radio sources, in contrast to their overall angular size.

The observations are taken from a recent survey of scintillating radio sources at 81.5 MHz which has been fully described elsewhere, and we also make use of some additional measurements at 151 MHz. Amongst the 1,500 sources listed in this survey is a complete sample of ~200 3CR sources, a substantial fraction of which have been optically identified and for which the redshifts are therefore known.

By restricting our attention to strongly scintillating sources (those which emit at least 40% of their 81.5 MHz flux from components having an angular size <2") which are also non-variable, we suggest that we obtain a more closely defined range of objects at large redshifts than has been available hitherto for use in the angular diameter versus redshift test. Our reasons are as follows:

(1) An investigation of the nature of this sample has shown that strong scintillators are sources of the highest intrinsic luminosity<sup>5</sup>.

(2) It may be deduced from the spectra of the sources, and by combining scintillation results with high resolution maps made with the Cambridge 5-km telescope, that for sources with steep spectra ( $\alpha > 0.75$ ) the compact features of size <1" are nearly always located in the outer components of typical 'double' radio galaxies. In addition, the ratio (component size/component separation) can be inferred for sources whose components are unresolved by mapping, and it is notable that the values inferred are in fair agreement with those obtained directly from mapping the larger sources. This shows that the source morphology is preserved over a wide range of angular size. The physical sizes of the compact features are typically 2-5 kpc and they are probably similar to the bright "heads" which have been found in Cygnus A (ref. 7); in strong scintillators, however, these regions must emit a substantial fraction of the total flux.

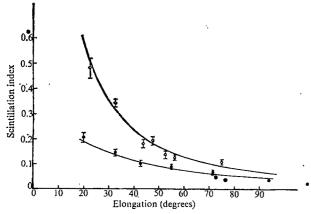


Fig. 1 The observed variation of scintillating flux density ΔS at 151.5 MHz as a function of solar elongation ε, for two typical sources. Also shown are theoretical curves for Gaussian sources of given angular size. (), 3C446 (0.1"); , 3C275 (0.7").

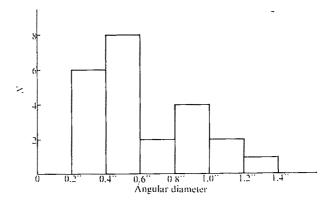


Fig. 2 The distribution of angular diameters for 23 strongly scintillating sources at 81.5 MHz.

(3) In a small number of cases strong scintillators are associated with objects having an overall angular size of less than 1", such as 3C273, 3C279 and 3C446. Sources of this type often exhibit temporal variability at centimetre wavelengths, and have flat radio spectra. These highly compact sources may therefore usually be distinguished, in the absence of angular size data, from the typical double radio sources which have steeper spectra and which are non-variable. Such sources have been excluded from our sample.

#### The observations

Measurements both of angular diameter, and of the fraction of the total flux in components of size <2", can be made with fair accuracy by the method of interplanetary scintillation on sources of high flux density, provided that they can be observed over a wide range of solar elongation ε. The method is illustrated in Fig. 1 which gives two examples of how the r.m.s. scintillating flux density  $\Delta S$ varies with  $\epsilon$ . The angular diameter is obtained by comparing the observations with theoretical curves based on a model of the interplanetary plasma8. The value is not sensitive to the assumed source model9 and the results that we use are based on a circularly symmetrical Gaussian model4. If two or more scintillating components of equal size, spaced by at least 1", are present in a given source (as might be the case for a typical double radio source) the derived angular size is unchanged but the fraction of the total flux in these components is slightly underestimated. The overall reliability of the scintillation technique has been checked by comparison with VLBI methods<sup>10</sup> at the high resolution limit of 0.1"-0.2" and by comparison with maps obtained with the 5-km telescope at the low resolution limit of 1"-2". We find no reason to doubt the validity of results such as those given in Fig. 1.

In the sample of 200 3CR sources there are (classified as A sources in Readhead and Hewish's catalogue') 23 strongly scintillating sources for which curves of a similar quality to those shown in Fig. 1 have been obtained. This sample excludes sources lying within ±20° of the galactic plane where interstellar scattering can give rise to a significant increase in the angular size at 81.5 MHz (ref. 11). The distribution of angular diameters derived from the scintillation curves is shown in Fig. 2.

The rapid decrease in the number of sources having sizes >1" is to be expected because the degree of scintillation falls steeply in this range. A more surprising feature is the peak in the distribution at about 0.4". This value is twice the resolution limit of 0.2" and is unexpected since, a priori, there is no reason to suppose that there will not be a very wide dispersion in the angular sizes of the compact components. If the actual sizes were more or less uniformly scattered over the range below 1.0" then we should expect the numbers to increase rapidly towards the resolution limit because the use of the scintillation technique

introduces a strong selection effect favouring the detection of sources having the smallest angular size.

What other observational selection effects might account for the observed distribution? One possibility might be that the compact components have roughly the same physical size and luminosity, and that they subtend an angle of 0.3"-0.4" when they lie at a distance such that their scintillating flux density falls near the limit for classification as an 'A' source in the catalogue. In that case, however, there should be a correlation between flux density and angular size which is certainly not observed. Thus this possibility cannot explain the observations.

A more important possibility is that we are measuring an apparent size due to angular scattering in the interstellar medium11. Our recent measurements (to be published shortly) of this effect show that an extragalactic point source would have an apparent angular diameter of  $0.17'' \pm 0.3''$  (sin  $b^{11}$ )<sup>-1/2</sup>, which gives a mean value of 0.2''for a random distribution of sources at latitudes  $b^{II} > 20^{\circ}$ . A further check was made by observing a number of sources in the sample at 151 MHz, where the interstellar scattering would be nearly four times smaller. At 151 MHz the angular diameters were typically only 20% smaller, as shown in Fig. 3, in good agreement with the above formula for source broadening at 81.5 MHz. It therefore follows that the values at 81.5 MHz are only slightly modified by interstellar scattering and this is not sufficient to produce a maximum in the distribution near 0.4". We conclude that the distribution of angular diameters is real and cannot be explained by observational selection or other spurious effects.

#### The angular diameter—redshift relation

The angular diameters of the scintillating components are shown as a function of redshift z in Fig. 3, where the limits of the scintillation technique are also indicated. When a given source was observed at 81.5 MHz and 151 MHz both values are shown, and the differences can be ascribed to interstellar scattering as mentioned earlier. It is not surprising, in view of the limitations of the technique, that the measurements show little obvious redshift dependence although there is a tendency for the mean diameter to increase at large z. There is, however, a remarkable deficit of sources having diameters less than about 0.3" in the range  $0.7 \le z \le 1.6$  (denoted by the quadrilateral in Fig. 3).

The sloping lines in Fig. 3 show the expected trend, on the basis of Euclidean geometry, for a population of sources whose physical sizes are independent of redshift. If the population in the range  $0.2 \le z \le 0.5$  is typical then we expect to find about six sources having diameters < 0.2" in the range 0.7 < z < 1.6. Instead of the expected number, clustered near the resolution limit of 0.2", no sources are observed, even though this is the region where the scintillation technique has the greatest detection sensitivity.

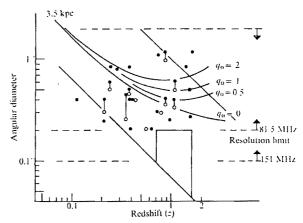


Fig. 3 The angular diameter *versus* redshift relation for strongly scintillating sources. ●, 81.5 MHz; ○, 151 MHz.

A straightforward explanation of these results can be given if a non-Euclidean world model is assumed. The curves in Fig. 3 show the angle subtended by a rigid rod of length 3.5 kpc for different cosmological models assuming  $H = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ . The observations do not seem to fit the curve for a deceleration parameter  $q_0 = 0$ , but are consistent with  $0.5 \le q_0 \le 2.0$ . Any tendency for the linear sizes of scintillating components to vary with epoch will, of course, modify the derived value of  $q_0$ . If  $q_0$  is to be smaller, for example, the linear size must increase with z.

It is clearly desirable to obtain information on a larger sample of sources but it is already evident that the angular sizes of scintillating components in radio sources are hard to explain by Euclidean geometry. Strongly scintillating radio sources appear to provide the best rigid rods that are currently available for cosmological tests.

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# letters to nature

# X rays predicted from flares of UV Ceti stars

Known UV Ceti flare stars are all located within 20 pc of the Sun, and their distribution seems to be isotropic. They are cool, low-luminosity objects with masses less than  $0.5 M_{\odot}$ . Both optical<sup>1</sup> and radio<sup>2</sup> radiations have been observed during flare events. X rays have also been predicted<sup>3,4</sup>, but no positive

observations have yet been reported. We present here new estimates of X-ray emission, based on the ratio between X-ray and radio flux from solar bursts. It is anticipated that the intensities of the X-ray flares will be more than 10 photons cm<sup>-2</sup> s<sup>-1</sup>; within the range of sensitivities of present satellite detectors (approximately 0.5-0.01 photon cm<sup>-2</sup> s<sup>-1</sup> for a flare duration of 3 min in the energy range 1-10 keV).

Table 1 Predictions and upper limits of X-ray emission during flares of UV Ceti stars

X-ray energy (keV)	Description	X-ray luminosity (erg s <sup>-1</sup> )	X-ray flux at Earth (photons cm <sup>-2</sup> s <sup>-1</sup> )
	Scaling model based on the ratio solar X-ray: sola	r radio (210 MHz)	
> 4.1	UV Cetf, 10 Jy (ref. 10)	$7.2 \times 10^{30}$	1
1.6-12.4		$1.5 \times 10^{32}$	40
0.62-1.6		$5 \times 10^{32}$	350
	Scaling model based on the ratio solar X-ray; sola	r optical (3.500-6.500 Å)	
>10	UV Ceti, $\Delta m_V = 1$ (ref. 4)	$3 \times 10^{24}$	$2 \times 10^{-7}$
1.6-12.4	, , , , , , , , , , , , , , , , , , , ,	$2.4 \times 10^{28}$	10-2
0.62-1.6		$1.8 \times 10^{28}$	10-2
>10	Model UV Ceti, $\Delta m_V = 1$ (ref. 3)	$3.4 \times 10^{31}$	2.6
>7.7	Measured 3 $\sigma$ upper limit, OSO-3 YZ CMi, $\Delta m_U = 1.5$ (ref. 15)	$3.6\times10^{33}$	300
•	Upper limits from measurements of the Galactic c	omponent of the diffuse	
	X-ray background and the assumed flare-star para		
7.7–12	(ref. 16)	$7 \times 10^{29}$	0.05
2–10	(ref. 17)	1031	3
0.2–2	(ref. 18)	$6 \times 10^{31}$	150
1-10	Minimum 3 $\sigma$ source detectability, MIT	1030	0.5
	OSO-7; UV Ceti flare of 3 min duration		

Variability in the types, intensities, and durations of stellar and solar flares makes uncertain both optical/X-ray and radio/ X-ray scaling comparisons. Uncertainties in the optical scaling model of Kahler and Shulman4 arise, however, from two additional sources. First, the optical excess from solar flares is difficult to measure and has been reported for only a few large flares. Second, because of the limited range of optical band widths in which flare stars have been observed, direct comparisons are not possible and bolometric comparisons between stellar and solar flare emissions are dependent on the model used. Predicting X-ray emission using the ratio between solar X-ray radiation and solar radio radiation is less ambiguous because radio signals from solar flares and from flare star bursts have been observed at the same frequencies. Scaling UV Ceti-type emission by using that ratio is justifiable because of the similarities between solar and flare-star bursts and because of the temporal correlation between the X-ray emission and the radio burst signals observed from solar flares<sup>5,6</sup>. Laboratory studies have shown the applicability of such scaling over remarkable ranges of the physical parameters7. Kahn<sup>8</sup>, using both plasma and synchrotron models, has shown that the observed characteristics of flares of UV Ceti stars are consistent with volumes, temperatures, and densities not much different from those suggested for the corresponding models of solar flares. Mullan9 has developed a model of the spots observed on red dwarf flare stars which indicates that the magnetic fields responsible for the star spots have strengths of approximately 20 kgauss. Such fields, approximately 10 times as strong as those observed in sunspots, would provide a factor of 100 in the energy available for release in flares over comparable volumes. The factor of 100 also helps to account, albeit only slightly, for the much greater radio luminosity of flare-star bursts relative to those of the Sun. Lovell<sup>2</sup> has pointed out that many flare-star radio bursts are 104-106 times more intense than the most intense solar radio flares.

Our predictions, based on the ratio between X-ray emission and radio emission from a particular solar flare<sup>10</sup>, are presented in Table 1 together with other predictions and observational upper limits. For comparable energy ranges, the X-ray to radio scaling results yield a factor of 10<sup>4</sup> more X-ray emission than the X-ray to optical scaling suggested by Kahler and Shulman<sup>4</sup>. The solar-flare data we have used does not quote a value for the flux above 10 keV; we have, however, estimated the X-ray emission for energies above 10 keV by scaling the predictions of Kahler and Shulman<sup>4</sup> by the factor of 10<sup>4</sup>. We

obtained an X-ray luminosity of  $3\times10^{28}$  erg s<sup>-1</sup> and an X-ray flux of  $2\times10^{-3}$  photon cm<sup>-2</sup> s<sup>-1</sup> at Earth for a 10 Jy radio burst (210 MHz) of UV Ceti. Although more optimistic than the predictions of Kahler and Shulman<sup>4</sup>, these values lie a factor of  $10^3$  below the predictions of Grindlay<sup>3</sup> and, in this energy range, outside the levels of detectability of present satellites.

Using the requirement that the integrated X-ray flux from all UV Ceti flare stars should not exceed the observed, diffuse X-ray background, and assuming the mean flare-star parameters cited by Edwards<sup>11</sup>, we have calculated upper limits for the X-ray emission of a  $\Delta m_V = 1$  flare of UV Ceti (Table 1). These values are 10-8 times the diffuse luminosity of the Galaxy in each energy range considered. The fluxes based on our scaling predictions do not exceed these limits by more than a factor of 10 in any of the energy ranges. Because of all the uncertainties in the flare-star parameters, especially in their Galactic distribution and density which have been determined only within 20 pc of the Sun, and because of the additional uncertainty in the frequency of occurrence of radio flare bursts, the disagreement is not significant. Depending on the details of their Galactic distribution, the UV Ceti flare stars may, in fact, satisfy the spatial, spectral, and flux characteristics required of unresolved sources of the observed, diffuse X-ray background12.

A search for coincident optical, radio, and X-ray coverage of flare activity in UV Ceti stars is now under way. As indicated by the final entry in Table 1, the MIT OSO-7 X-ray detectors have sufficient sensitivity to refute or to confirm the more optimistic predictions if coincident coverage is obtained. Rates of radio flare bursts above a threshold of 0.2 Jy are comparable to the rates of optical flares above a threshold of approximately  $\Delta m_U = 0.5$  for YZ CMi and AD Leo<sup>13</sup>. A coordinated optical and radio observing programme, using high time-resolution photometric techniques (see ref. 14) has been carried out; and we are examining the OSO-7 data for coincident X-ray observations. Improvement in X-ray sensitivity by a factor of 10 can be obtained using the new, pointed X-ray observatories such as the Netherlands Astronomical Satellite (ANS) or the MIT SAS-C observatory which is scheduled to be launched next June. Prospects for coordinated observing programmes are being investigated.

In the case of solar fast-drift radio bursts (Type III), low energy (~4 keV) X-ray emission is more strongly correlated with the decimetre radio flux than with the metre radio flux, although considerable scatter exists in both populations

(S. Kahler, personal communication and ref. 19). Still better correlation has been found between the higher energy (>20 keV) X-ray flux and the 3 GHz radio flux observed in impulsive solar-flare events20.

Unfortunately, radio observations of UV Ceti flare stars have been almost entirely at radio frequencies about or below 400 MHz. We hope that higher frequency radio observations will be possible and will soon be available.

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### Positional agreement between 3U1706+32 and the cluster of galaxies A2241

UNIDENTIFIED X-ray sources at high galactic latitudes have been the subject of much discussion (see ref. 1 and refs therein). We call attention here to the existence of a rich Abell cluster of galaxies in the error box of the Uhuru high galactic latitude source 3U1706+32. The X-ray error box is rather large (9.8 square degrees), and the apparent intensity of the source is  $R_x = 4.1 \pm 0.6$  counts s<sup>-1</sup> in Uhuru counts<sup>2</sup>. The cluster, Abell 2241, is located at 16 h 57.8 min  $+32^{\circ}37'(1950)^{3}$ ; it belongs to distance group 3 and has an estimated redshift,  $z \approx 0.07$ (ref. 3). The cluster is of richness group 0 (ref. 3) and has been classified4 as an irregular (I) cluster. The positional agreement between this cluster and 3U1706+32 was not reported in the survey of positional correlations between 3U X-ray sources and Abell clusters of galaxies of distance groups,  $D \leq 3$ , (ref. 5). The cluster A2241 should, therefore, be added as a possible identification to the list<sup>5</sup> of 21 clusters which have been tentatively identified with X-ray sources; no additional Abell cluster of distance group 3 or nearer lies within Uhuru<sup>2</sup> high galactic latitude error boxes.

Although A2241 is located within the error box of 3U1706-32 and is therefore a candidate for identification with the X-ray source, we stress that a much improved X-ray position is required before a certain identification can be made. The X-ray luminosity of the cluster, assuming it to be at a redshift of 0.07, is about  $10^{45}$  erg s<sup>-1</sup> (for  $H_0 = 55$  km s<sup>-1</sup> Mpc<sup>-1</sup>). This luminosity is somewhat higher than the X-ray luminosity range ( $\approx 10^{43}$  to  $\approx 3 \times 10^{44}$  erg s<sup>-1</sup>; see ref. 6) obtained for the other irregular clusters previously identified with X-ray sources, but is comparable with the luminosities of the brightest known regular X-ray clusters.

As is true for most of the other X-ray clusters, radio emission has also been observed from A2241; a double radio source, with a strength of 0.9 Jy at 1.445 MHz, is located in the projected area of the cluster. An elliptical galaxy is located at the radio centroid7. It will be very interesting to learn whether or not the X-ray source is centered on this galaxy.

We consider that A2241 is a possible optical candidate for the high galactic latitude X-ray source 3U1706-32. A better measurement of position is however, required. It is also important to measure the cluster redshift so that, if the identification is correct, the X-ray luminosity can be calculated more accurately.

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#### Radio structure of the M87 jet

THE central region of M87 has a complex radio structure. There are at least three components1.2, with diameters  $\approx 0''.0013$ ,  $\approx 0''.01$  and  $\approx 0''.3$ , coincident with the optical nucleus of the galaxy and on either side there are two elongated components, ~25" in extent, the radio counterparts of the jet and counterjet3,4. When observed with a resolution of ≈5", the structure of these outer components is similar and depends little on observing frequency, but at higher resolutions the jet component is seen to possess fine structure which the other lacks5.6. Here I report results of interferometer observations at 408 and 1,666 MHz, with resolving powers  $\approx 0.5^{\circ}$ along the jet, which reveal the position and extent of this fine structure in more detail.

At 408 MHz the interferometer was formed from the Mk 1A (76 m) telescope at Jodrell Bank and a 25-m parabaloid at RRE Defford (baseline 172,700 $\lambda$ ). At 1,666 MHz the Mk II (38 m $\times$ 25 m) telescope and the Mk III (38 m×25 m) telescope were

Table 1 Parameters of the models fitted to the observations of M87							
a 1,666 MHz Component separation	Position	Half-power	Half-power	Position angle of	Flux		
from nucleus (") 0.0*	angle (degree)	length (") U*	width (") U*	elongation (degree)	density (Jy) 3.5*		
$0.0*$ $9.1 \pm 3.0$	$289.6 \pm 3.0$	$0.6 \pm 0.3$ 5.5 $\pm 3.0$	U* U*	30030 2925	1.5 == 0.5 1.8 = 0.6		
$12.5 \pm 0.3$ $14.4 \pm 0.2$ $17.6 \pm 0.2$	$290.5 \pm 0.3$ $289.6 \pm 0.3$ $292.0 \pm 0.5$	$1.5 \pm 0.3$ $1.4 \pm 0.3$ $2.3 \pm 0.3$	U* U* U*	288 ± 10 282 <u>=</u> 10 298 <u>±</u> 10	$2.8 \pm 0.5$ $1.4 \pm 0.3$ $1.5 \pm 0.3$		
5 408 MHz							
$0.0*$ $8.2\pm0.4$ $12.3\pm2.0$ $12.3\pm0.3$	291.6±0.4 287.0±4.0 290.7+0.5	$0.5\pm0.2$ $1.8\pm0.4$ $9.0\pm3.0$ $\lesssim 0.5$	U* U* U* U*	$260 \pm 20$ $304 \pm 10$ $300 \pm 20$	$4.6 \pm 0.5$ $1.6 \pm 0.5$ $2.7 \pm 1.0$ $1.0 \pm 0.2$		
14.5±0.2 17.6±0.6	$289.6 \pm 0.3$ $291.6 \pm 0.4$	$0.8 \stackrel{\sim}{\pm} 0.3$ $1.6 \pm 0.3$	U*	$290 \pm 15$ $260 \pm 15$	0.8±0.2 0.7±0.2		

<sup>\*</sup> Held constant during the analysis.

used (baseline 132,300 $\lambda$ ). The 1,666-MHz interferometer incorporated an improved radio link system (Spencer and Warwick, unpublished) which compensates for changes in the link path delay due to changes in atmospheric conditions. The resulting phase stability enables the position of the radio nucleus to be measured; it is within 0.5" in right ascension and 2" in declination of the optical nucleus, in agreement with earlier radio position measurements.

Strip distributions in right ascension, obtained by direct Fourier inversion of the 1,666 MHz amplitude and phase data,

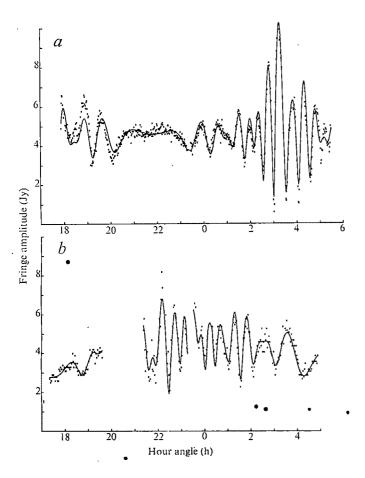


Fig. 1 Variation of fringe visibility with hour angle at a, 1,666 MHz; b, 408 MHz.

show that there are other compact components in the source. The two which are best defined lie on the jet side at between 12" and 15" and at  $\approx$ 18" away from the nucleus. On the other side the amount of emission from compact components is smaller but there may be weak features (  $\lesssim 0.5$  Jy) at  $\approx\!8^{\prime\prime}$  and  $\approx\!13^{\prime\prime}$  away from the nucleus. To investigate the radio structure further the fringe amplitude data were analysed using a model-fitting technique which assumes that the components have Gaussian symmetry. The data are shown in Fig. 1a with the curve which would be given by the adopted model brightness distribution. Because the range of resolutions in the N-S direction is poor, at the declination of M87, the widths of the components are not well defined. In the adopted model all the widths were constrained to be zero but values up to  $\approx 2''$  are also consistent with the data. The values of the optimised parameters of the model are given in Table 1.

The 408-MHz data were only analysed using the model-fitting technique and the adopted values of the parameters are also given in Table 1. The absolute positions of the components cannot be determined at this frequency but from the agreement between the two models it is clear that the 408-MHz observations refer to the same regions of the source as those at 1,666 MHz and that the most intense component is in fact the radio nucleus

The spectra of the individual sources in the nucleus cannot be determined unambiguously. Graham's attempt<sup>3</sup> is incorrect because he recognised only two components and he also included some flux density measurements which are almost certainly contaminated by contributions from compact components in the jet. The present work shows that at least one of the very compact nuclear sources must have a low frequency cut-off near 1 GHz, for the unresolved component in the model has a flux density of 3.5 Jy at 1,666 MHz but less than  $\approx$ 2 Jy at 408 MHz. Further high resolution observations are needed to delineate the spectra more clearly.

The agreement between the well defined components of the 408 and 1,666 MHz models means that the basic structure of the radio jet depends little on observing frequency. The other components in the two models do differ but at both frequencies they indicate that the region between the nucleus and the well defined components in the jet is also a source of radio emission. The brightness distribution of this region is probably complex and synthesis observations are required to reveal its structure unambiguously. Figure 2 shows a photograph of the central region of M87 (ref. 11) and below, plotted on the same scale, contours of the radio emission as described by the model at 1,666 MHz. The striking correspondence between the brightness distributions at optical and radio wavelengths indicates that the structure of the jet is essentially the same over a frequency range of  $\approx 10^6$ :1.

U, Unresolved, dimension arbitrarily put to zero.

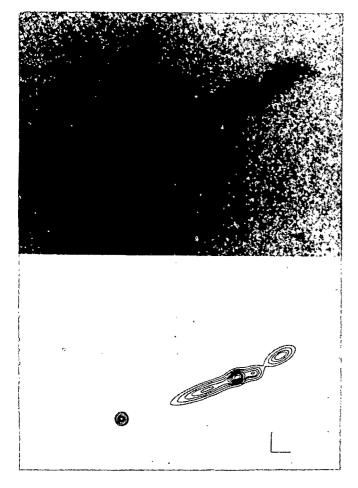


Fig. 2 Comparison of the optical and radio structures of the M87 jet. The photograph is taken from Felten et al. 11. North is at the top and east to the left. Many contours have been omitted from the intense nuclear source for the sake of clarity and the poorly defined component widths have been arbitrarily assigned the value 1". The L in the bottom right hand corner represents 2" in each coordinate. (Photograph reproduced with permission of the Astrophysical Journal.)

A convincing explanation of the physics of the M87 jet is still awaited. The present results show clearly that the electrons responsible for the radio and optical synchrotron emission have very similar spatial distributions, but further high resolution work, especially to locate the variable component<sup>12</sup> and to determine the polarisation structure of the knots at different frequencies, is required before the radio observations can place important constraints on theories of the jet.

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## 22.2-GHz high emission nebulosities associated with the Carina Nebula central region

MAPS of Carina Nebula, obtained at relatively low frequencies, have shown that most of the radiation arises from a central region and has a simple double structure, separated by about 9.4'. Gardner et al.1 studied Carina Nebula at 5 GHz, the highest frequency so far used, with the Parkes radio telescope and observed a beamwidth of 4'.

Shaver and Goss<sup>2</sup>, used the Molonglo interferometer at 408 MHz (with 2.65' and 2.86' beams in right-ascension and declination, respectively) to study the same region. The two main sources, G287.4-0.6 and G287.6-0.6 (Car I and Car II, respectively1) were suggested as optically-thin thermal regions in the range 408 MHz to 5 GHz, and were assigned electron temperatures of 8,600 K and 8,100 K, respectively.

Using the 13.7 m radio telescope of the Itapetinga Radio Observatory, Atibaia, SP, Brazil, we derived a continuum map at 22.2 GHz from the central region of Carina Nebula, with a beamwidth of 4', and a linearly polarised feed. Because of the large angular extension of the source, we used a loadswitching and on-off technique, with beams 45' apart, for the mapping. Successive positions measured in the source were separated by one beamwidth in declination and/or rightascension. We corrected for tropospheric attenuation and calibrated all measurements against Virgo A, for which we

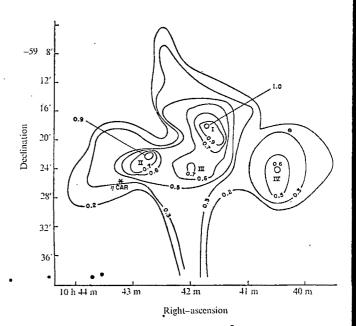


Fig. 1 A radio map from the central region of Carina Nebula obtained on a continuum at 22.2 GHz. Isotherms are normalised in relation to the stronger emission at Car I.

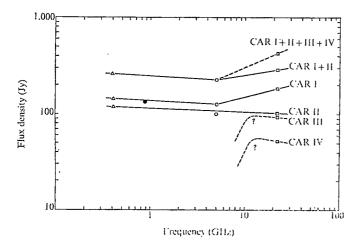


Fig. 2 Integrated spectra for the different components in Carina Nebula. At 22.2 GHz, the flux densities are: 183 Jy for Car I; 104 Jy for Car II; 94 Jy for Car III and 52 Jy for Car IV (all within  $\pm 20\%$ ).  $\triangle$ , ref. 2;  $\bigcirc$ , ref. 3;  $\square$ , our data.

assumed a flux density of 21.5 Jy at 22.2 GHz. The inaccuracies of the intensities measured from Carina Nebula were estimated to be about  $\pm 20\%$ , as the sum of r.m.s. errors, attenuation errors, and source size correction errors. The absolute pointing accuracy of the radio telescope is known to be better than 20", which is negligible compared with the beamwidth at 22.2 GHz.

Figure 1 shows the map at 22.2 GHz, not restored for aerial smoothing. The two main sources identified at 5 GHz1.3 agreed well with those at 22.2 GHz, but the whole region became rather more complex at this higher frequency. Two new structures appeared, one at about  $\alpha_{1950.0} = 10h$  42.0min and  $\sigma_{1950.0} = -59^{\circ}$  24.0', and another at  $\alpha_{1950.0} = 10h$  40.4min and  $\sigma_{1950.0} = -59^{\circ}$  24.0' (corresponding to G287.5–0.7 and G287.2-0.8, respectively) which we have designated Car III and Car IV, respectively.

No enhanced radiation was measured in the direction of ηCar itself, and the radio emission from the central region seemed to follow southwards in a straight bridge centred at about  $\alpha_{\text{1950.0}}=10\text{h}$  42.0min. There is generally no good correspondence between the radio features at 22.2 GHz and the optical features from the nebula.

Figure 2 shows spectra for the central region of the nebula, using data from Gardner and Morimoto3, and Shaver and Goss<sup>2</sup>. We restored the sizes of the sources by simplified techniques<sup>4,5</sup>; to derive integrated fluxes at 22.2 GHz and we assumed a Gaussian form for the antenna beam, and Gaussian distribution of brightness across the sources, with elliptical symmetry. Only Car II seemed to be a normal H II region, optically thin in the range 408 MHz to 22.2 GHz. The other spectra, the integrated ones, and that one for Car I, seem to receive contributions from sources with high emissions, still optically thick at 5 GHz, and probably thin at 22.2 GHz.

The two identified and resolved new sources, Car III and Car IV may be very compact H II regions, with turnoverfrequencies in the range 5 to 22.2 GHz. If we assume that these sources are already optically thin at 22.2 GHz, and the turnover frequency  $f_c$  is about 10 GHz, we can estimate a mean emission after Terzian and Dickey<sup>6</sup>

$$\langle E \rangle = (f_c^2 T_e^{3/2})/k_c \tag{1}$$

where  $T_{\rm e}$  is the electron temperature, and the absorption coefficient  $k_c$  at the turnover frequency (MHz) is

$$k_c = 9.8 \times 10^{-15} \ln(49.5 T_e^{3/2})/F_c$$
 (2)

Assuming that  $T_e \approx 8,600 \,\mathrm{K}$  for these sources, we obtain high mean emissions, of about  $4.3 \times 10^8$  cm<sup>-6</sup> pc.

An accepted distance of Carina Nebula is about 2.7 kpc (ref. 7), from which we estimate an approximate radius for Car IV (better defined in Fig. 1) of about 3.2 pc. The value for the emission is

$$\int_0^R N^2 \, \mathrm{d}R \tag{3}$$

where R is the radius and N the electron density. For a constant distribution of N along R, and along the line of vision, we find an electron density of about  $1.2 \times 10^4$  cm<sup>-3</sup>. The values for  $T_e$ ,  $\langle E \rangle$  and N, estimated for the enhanced sources in the central region of Carina Nebula, correspond to an extreme case of a typical compact H II region as proposed by Mezger<sup>8</sup>. Measurements at a frequency between 5 and 22.2 GHz, and at a frequency above 22.2 GHz, are required to understand better the nature of this interesting nebula.

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## Gyron Field—gravitational analogue of magnetic force

An electrostatic field E in a stationary coordinate system, when referred to a moving coordinate system, can produce both an electric field E\*, and a magnetic field B\*. If v is the uniform velocity of motion, perpendicular to E, then by the usual Lorentz transformation:

$$\mathbf{E}^* = \mathbf{E}/\sqrt{(1-\beta^2)}$$
 and  $\mathbf{B}^* = \mathbf{v} \times \mathbf{E}/c\sqrt{(1-\beta^2)}$ , where  $\beta = \mathbf{v}/c$ .

Consider two stationary point masses,  $m_1$  and  $m_2$ , with respective electric charges  $q_1$  and  $q_2$ . Suppose that the particles are located at 0, r/2, 0 and 0, -r/2, 0 in a system of Cartesian coordinates. If the masses and charges are chosen so that the force of electrostatic repulsion exactly balances that of gravitational attraction, then

$$q_1 q_2 / 4\pi \varepsilon_0 r^2 - G m_1 m_2 / r^2 = 0 \tag{2}$$

where G is the constant of gravitation and  $\varepsilon_0$  is the electrical permittivity of space. Positive signs indicate repulsion and negative signs attraction.

Now suppose that the two particles, while maintaining the same distance apart, are moving with uniform velocity, v, parallel to the x axis. This motion will not disturb the equilibrium of the particles.

From equation (1), the electric force of particle 1 acting on particle 2 becomes

$$\mathbf{E}^* q_2 = q_1 q_2 / 4\pi \epsilon_0 r^2 \sqrt{(1 - \beta^2)}$$
 (3)

Similarly, the magnetic force becomes:

$$q_{2}(\mathbf{v} \times \mathbf{B}^{*})/c = -(q_{2}/c^{2})\mathbf{v} \times (\mathbf{v} \times \mathbf{E})$$

$$= -q_{1}q_{2}\beta^{2}r/4\pi\epsilon_{0}r^{3}\sqrt{(1-\beta^{2})}$$
(4)

The negative sign indicates that this component of force represents attraction.

The electric charges  $q_1$  and  $q_2$  are invariant under a Lorentz transformation, whereas the masses  $m_1$  and  $m_2$  must vary as

$$m_1^* = m_1/\sqrt{(1-\beta^2)}$$
, and so on (5)

Within the framework of special relativity, the ordinary gravitational force, analogous to equation (3) in the moving system, becomes:

$$Gm_1*m_2*/r^2\sqrt{(1-\beta^2)}=Gm_1m_2/r^2(1-\beta^2)^{3/2}$$
 (6)

Hypothetically, linear motion of translation will not disturb the equilibrium of the two point masses. The gravitational force in equation (6) is, however, not functionally compatible with the total electric forces of equations (3) and (4). To achieve the balance of forces a velocity-dependent gravitational force analogous to the magnetic component of equation (4) (which is proportional to  $\beta^2$ ) must be added to equation (6). Thus:

$$[q_1q_2/4\pi\varepsilon_0r^2\sqrt{(1-\beta^2)}](1-\beta^2)-[Gm_1m_2/r^2(1-\beta^2)^{3/2}]$$

$$(1-A\beta^2)=0 \quad (7)$$

where A is a parameter to be determined. From equation (2):

or 
$$A\beta^2 = 1 - (1 - \beta^2)^2;$$
 
$$A = 2 - \beta^2$$
 (8)

This analysis seems to establish the existence of a gravitational force dependent on the relative velocities of moving bodies, as well as on their masses. We suggest that this special force, which must apply to translational motion as well as to rotational motion, be called the 'Gyron Force'.

Lorrain and Corson<sup>1</sup> have mentioned the possibility that such a force exists. They have speculated about "a gravitational equivalent of the magnetic field" but have given no quantitative statement. Moreover, they indicated that this force "could only be attractive", whereas the positive sign of A in equation (7) indicates that the force, as we have derived it, is repulsive. Furthermore, Blokland<sup>2</sup> has concluded, by somewhat obscure reasoning, that the quantity we have designated as A should probably have a value of about 2.

In special relativity, the apparent mass of a body depends on its energy, in terms of the familiar Lorentz transformation. Precise experiments<sup>3</sup> have shown that gravitational and inertial masses are identical to at least 13 significant figures. Apparent mass rather than rest mass must, therefore, be used to define gravitational forces. Thus, our derivation of the gyron field disagrees with that of Sciama4 who used the rest mass instead.

This analysis ignores such phenomena of general relativity as curvature of space. It is, however, clear that the principle must apply in the area common to both general and special relativity. We recognise that a complete derivation must be built on a tensor rather than a vector or scalar theory of gravitation. The result derived here does not depend, however, on any specific theory of gravitation.

We further point out that the existence of forces associated with moving or, especially, rotating masses strongly supports the idea that gravitational waves exist. Such waves must be quadrupole in character. Only the existence of electric charges of opposite sign makes possible the radiation of dipole electromagnetic waves.

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# Observation of Comet Kohoutek at 1.4 mm

We made observations of Comet Kohoutek at Kitt Peak with the National Radio Astronomy Observatory (NRAO) 36-foot telescope during two consecutive periods: December 27, 1973-January 2, 1974; and January 6-January 13, 1974.

We used a 1-mm system with our own high performance three-part bolometer<sup>1</sup>: diameter=6.5 mm;  $\tau \simeq 7$  ms; optical NEP at 1 mm=5×10<sup>-14</sup> WHz<sup>-1</sup>. It has a limit of detection of 2 Jy per hour of observation on the radiotelescope with good weather. The system applies a differential modulation at 20 Hz on the sky with two beams each of 1' separated by 2.2'. Scanning of the source and secondary low-frequency modulation to eliminate drifts is carried out by moving the whole telescope.

The bandpass of the system ranges from 1 mm to 3 mm, with a sharp cutoff at 1 mm; the efficiency increases from about 25% at 1.2 mm to 100% at 2 mm. The mean wavelength on a planet of spectral index 2 is thus about 1.6 mm. In the case of cometary dust emission, with a spectral index of pro-

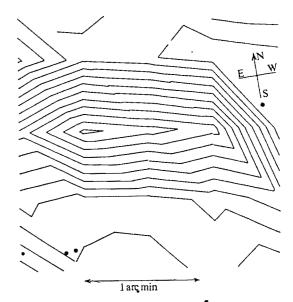


Fig. 1 Comet Kohoutek December 30, 1973; differential isoflux at 1 mm. This map shows interpolated values of points separated 0.5'. It is obtained from preliminary data taken from 1855 to 2000 ur, which are not corrected for field rotation or for atmospheric drift.

bably between 3 and 4, we estimate the mean wavelength to be 1.4 mm.

The field of view of the instrument was of the order of 1' quasicircular, determined from observations of unresolved sources such as planets. The absolute calibration of the flux was made through a straightforward comparison between the observed cometary emission and the flux coming from different planets, observed through the dome during the same day (J.D.G.R. et al., unpublished), corrected for the atmospheric attenuation given by the relationship  $\exp(-a \sec z)$ , where a is a function of time and z is the zenith angle. The position of Venus and Jupiter very close to the comet made this calibration particularly easy.

During the first period, Comet Kohoutek (1973f) was detected clearly on December 30, 1973. On the first evaluation, the cometary flux was of the order of  $100\pm30$  Jy. It was even possible to map the comet's head (Fig. 1). In spite of the poor spatial resolution, it is quite clear that this shape is elongated and orientated in the direction of the Sun, demonstrating that it was the comet, which was under observation.

We next observed the comet on January 12, 1974, when it was much fainter: its flux was then of the order of  $10\pm5$  Jy. This result, compared with the first, shows that to a first approximation the cometary flux at a wavelength of about 1.4 mm seems to decrease as  $r^{-2}$ , where r is the distance from the comet to the Sun.

The total energy available for heating the dust grains is given by:

$$E_1 = \sigma T^4 \sim 1/r^2$$

Therefore, the temperature of the grains should change as  $r^{-1/2}$ . The observed decrease in flux density is much more rapid than anticipated, implying a decrease in the global emissivity of the dust cloud.

This decreasing rate of emissivity may arise from several sources. Two likely possibilities are, first, a change of the dimensions of the emitting head (that is, a probable change in the production rate of dust); second, a change in the emissivity of the dust grains.

Consider the first hypothesis, and suppose that the flux should change as  $\Delta^{-2}r^{-1/2}$ , where  $\Delta$  represents the distance between the comet and Earth. It can then be calculated, to fit the observed variation ( $\Delta^{-2}r^{-2}$ ) to the predicted variation, that the size of the head changed at that wavelength from roughly 1' at the end of December to about 20" around January 12, 1974.

This observation, which represents the first detection at 1.4 mm of a cometary emission, provides new information concerning the rate of dust production and the ejection mechanism of the dust. Comparison of this observed flux with others made at shorter and longer wavelengths, using the blackbody emission law, will probably give the dependence on wavelength of dust grain emissivity, which is directly related to their composition and size.

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# Microwave search for molecules in Comet Kohoutek

We report here a molecular radio line search in Comet Kohoutek using the 108-116 GHz spectral line receiver on the NRAO 36-foot telescope during January 14-18, 1974. We discuss some of the reasons for the negative results obtained in our search for transitions of methyl cyanide, cyanogen, cyanoacetylene, propynal and formic acid (all good 'parent' molecules for the diatomics and radicals known in the optical spectra of comets).

We particularly searched for the two transitions of methyl cyanide which had been detected by Ulich and Conklin¹ when the comet was 0.8 AU from the Sun before perihelion. As we were observing the comet after perihelion, when it was approximately the same distance from the Sun, the temperature of the cometary material and the excitation conditions should have been comparable. The transitions were detected at the level  $T_A \sim 0.6 \pm 0.1$  K, and our peak-to-peak upper bound for the transitions was  $T_A \sim 0.3$  K.

Initially, we thought that this might indicate a time dependence in the sublimation of 'parent' molecules, perhaps caused by inhomogeneities in the composition of the comet nucleus. We have since learned, however (T. Clark, private communication) that there was a 'time of flight' error in the position ephemeris of the comet that we and many other radioastronomers used. During our observations, the magnitude of the error was ~ 45", as it was during the observations of Ulich and Conklin who used the same ephemeris. For observations with beamwidth 1', a pointing error of this magnitude is clearly very serious. It seems unwarranted therefore to draw many conclusions about the existence of molecules in the comet from these observations.

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#### Solar neutrino puzzle

Davis and his collaborators, using a technique based on the reaction  $^{37}\text{Cl}(v,e^-)^{37}\text{Ar}$ , have been able to set a bound to the flux of neutrinos arriving from the Sun. The latest experimental results¹ correspond to 1 SNU (solar neutrino unit,  $1\times10^{-36}$  neutrino capture per  $^{37}\text{Cl}$  nucleus per second), whereas solar models yield values higher by one order of magnitude². Here I propose an explanation of the discrepancy between theory and experiment on the basis of the assumption that strong interactions between nuclear particles are of gravitational nature³-6.

I shall assume that within the range of nuclear forces, the gravitational constant is 38 orders of magnitude larger. For

distances larger than the Compton wavelength of the proton  $\lambda = 2.1 \times 10^{-14}$  cm, its value decreases exponentially, not to zero, but rather to a value of the gravitational constant as we know it. On the other hand, for distances within 0.21 fermi, the gravitational force would behave as a short range, chargeindependent, non-exchange force, responsible for the strong interactions between nucleons. To remain in agreement with the mass defects of atomic nuclei, the value of the gravitational constant at distances which are small compared to the wavelength of the proton has to be of the order  $G^* = 2.75 \times$ 10<sup>30</sup> c.g.s.

The assumption of a limiting range in the gravitational potential is in itself not as arbitrary as it may seem. The Hubble diagram of quasars7 suggests that the cosmological constant is not zero, which represents by definition a limiting range of the gravitational field. I suggest that there is also a lower boundary. Our physical world is observable between ~ 4,000 Mpc and 1/5 fm.

Should strong interactions between nucleons be gravitational, then all signal carriers with zero rest mass, such as photons and neutrinos, would interact with nucleons like hadrons if their energy is a few GeV. This has been observed for photons in experiments at DESI, Hamburg. An increase of the interaction forces with increasing gravitational mass of the interacting particles could also explain the unexpected increase in the total proton-proton cross section obtained with the CERN intersecting storage rings by the Pisa and Stony Brook teams at energies above 20 GeV.

At  $G^* = 2.75 \times 10^{30}$  c.g.s., the gravitational radius of the proton will become of the order of the Compton wavelength of the proton, leading to the inference that elementary particles behave as black holes. A hypothetical observer within such a black hole can use the Friedmann field equations. Cosmological entities observable within this 'lilliputian' universe have nuclear physical counterparts for an outside observer. For instance, the absolute value of the cosmological constant within the lilliputian universe is identical with the Compton wavelength of the particle. Because of the large value of  $G^*$ , the proton will occupy a hypersphere of radius  $\sim \lambda$  embedded, as is our world, in a four-dimensional continuum. Should the hypersphere of the proton intersect the hypersphere of our world, a proton will appear in our world, as a closed twodimensional space of constant curvature. (Antiprotons intersect our three-dimensional space from the other side.) Assuming that the hyperspheres of particles cannot intersect each other, we arrive at the concept of the hard core, with a range of  $\sim 2\lambda = 0.42 \times 10^{-13}$  cm, which is about the range of the hard core,  $0.53 \times 10^{-13}$  cm, in Levy's potential of nuclear forces. Cosmological, quantum mechanical laws and geometrical rules of two- to four-dimensional spaces supply a sufficient number of equations to yield a quantitative model-like description of fundamental elementary particles<sup>3-5</sup>.

A gravitational interaction between neutrinos and nucleons leads to the inference that even radioactive neutrinos, or neutrinos produced in nuclear reactions occurring in the interiors of stars, can transfer a momentum to nucleons and lose energy while passing through the mass of the star. As long as the transferred energy is small compared to the rest mass energy of the target particle, the momentum transfer can be computed in a classical manner. A neutrino of relativistic mass  $E_{\nu}/c^2$ , passing at a distance a from a nucleus of mass m, will through gravitational interactions transfer a momentum:

$$p_a = aE_v 2G^* m \lambda^{-1} c^{-3} \int_{a}^{\infty} r^{-2} (r^2 - a^2)^{-1/2} (1 + r) \exp(-r) dr$$

where distances are expressed in units of the Compton wavelength of the proton,  $\lambda = 2.1 \times 10^{-14}$  cm. The energy loss of the neutrino will be

$$\Delta E = p_a^2/2m \text{ erg}$$

This energy loss, occurring in an interaction with a nucleus of atomic weight A, can be approximated by

$$\Delta E = 1,630 A E_v^2 \exp(-4.10a) \qquad 0.5 < a < 1.0$$
  
= 390 A E\_v^2 \exp(-2.66a) \quad 1 < a < 5

Assuming that in such elastic interactions a neutrino cannot approach closer to a proton than the hard core range  $a = 2r_0 = 0.625$ , and energy losses at impact parameter a > 5 are negligible, the energy loss suffered by a neutrino of energy  $E_0$  MeV after passing through an absorber of unit (1 g cm<sup>-2</sup>) thickness will be

$$dE/dR = 8.7 \times 10^{-8} E_{y}^{2} \text{ MeV}$$

After traversing an absorber of R g cm<sup>-2</sup> thickness a similar neutrino will emerge with an energy

$$E_{\nu} = E_0/(8.7 \times 10^{-8} R E_0 + 1) \text{ MeV}$$

I note that this result is insensitive to the chemical composition of the absorber. (Inelastic collisions are not considered.)

The average density of the Sun being 1.41 g cm<sup>-3</sup>, and its radius  $5.4 \times 10^{10}$  cm,  $R = 7.6 \times 10^{10}$  g cm<sup>-2</sup>. A neutrino starting with an energy  $E_0$  MeV at the centre of the Sun will leave it with energy  $E = E_0/(6.57 \times 10^3 E_0 + 1)$  MeV. A neutrino from either the <sup>7</sup>Be reaction ( $E_0 = 0.861$  MeV), or the <sup>8</sup>B reaction  $(E_0 = 14.06 \text{ MeV})$  would arrive at the Earth with energy ~150 eV, far below the threshold (0.81 MeV) needed to detect neutrinos in the Homestake mine experiments of Davis.

The energy loss would, however, be much less if the neutrinos were to originate in sunspots, or in stars with densities much below the density of the Sun. One would expect Davis to observe some neutrino flux through the 37Cl(v,e-)37 Ar reaction during sunspot activity, or perhaps during outbursts of nearby supernovae (see ref. 8 for example).

My hypothesis could be tested at an accelerator where neutrinos and antineutrinos of energy 0.5-5 GeV are produced. According to my ideas, some of their energy would be lost in the iron shield used to absorb the muons and other ionising particles. A neutrino of energy 1 GeV, after traversing an iron absorber 20 m thick, would emerge with an energy of 700 MeV, adequate to produce muons when entering a bubble chamber.

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# Elemental abundances in interplanetary dust

It is generally believed that the main fraction of dust in the interplanetary medium is generated by the gradual disintegration of short-period comets in the inner Solar System1,2. Analysis of interplanetary dust-grains is, accordingly, a technique for gathering information on the nature of cometary matter. Until now, however, no particle with

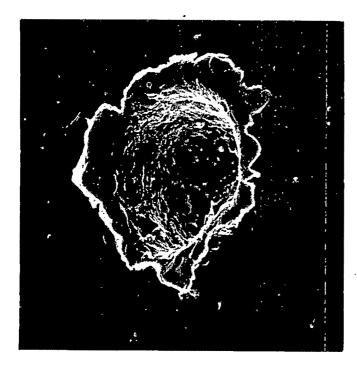


Fig. 1 110 μm diameter micrometeorite crater from Skylab-IV.

an unarguable interplanetary origin has been subjected to detailed chemical analysis.

Here we report the measurement of elemental abundances in two interplanetary dust grains obtained by analysis of meteoroidal residue found inside micrometeoritic craters. The two craters were discovered by scanning optically the 800 cm<sup>2</sup> aluminium surface of the S-228 transuranic cosmicray experiment, exposed to space for 67 d during the Skylab-IV mission. The larger crater is 110 µm wide (measured at the intersection of the pit with the plane of the original surface) and 75  $\mu$ m deep (measured from the pit bottom to the original surface plane). The smaller crater is 35  $\mu$ m wide and 26  $\mu$ m deep. The morphology of both craters is identical to hypervelocity craters produced in laboratory simulations and to craters found on metal grains from lunar soils3. The morphology is diagnostic proof that the craters were produced by genuine interplanetary dust grains impacting at velocities in excess of a few km s<sup>-1</sup>. Laboratory calibrations on aluminium targets (J. F. Vedder, private communication) indicate that the meteoroids had diameters of 30  $\mu$ m and 9  $\mu$ m.

In the scanning electron microscope, residue of the impacting meteoroids can be seen as conspicuous blebs and stringers lining the walls and floors of the craters (Figs 1 and 2). Energy-dispersive X-ray analysis of the 110  $\mu$ m crater in the SEM revealed the presence of Fe, Mg, Si, Ca and Ni in the crater bottom with relative peak heights qualitatively similar to those obtained for chondritic meteorites. For a more quantitative result, both craters were analysed in an ARL-EMX electron microprobe. The craters were tilted with respect to the electron beam and spectrometer to eliminate blocking of the emitted X rays by the steep crater walls. The sizes of the spots analysed on the floors of the large and small craters were 30  $\mu$ m and 3  $\mu$ m respectively. Only a single spectrometer was used for all elements, thereby eliminating the differential geometry problems that would arise from using different spectrometers for different elements. During an analysis run, the orater was kept in a fixed position and the spectrometer was scanned over all of the elements analysed. Before and

after the analysis runs, the spectrometer was scanned over mineral standards for calibration. Repeatability of the peak measurements on the standards was good, and errors arising from spectrometer tuning were small compared with errors caused by the geometry of the crater bottom. Two runs were carried out on each crater. The beam was repositioned and refocused between runs. The aluminium substrate material round the craters was pure; the only indigenous elements detected in it were Ti (130 parts per million), Mg (300 p.p.m.) and Si (4,000 p.p.m.). The only significant substrate interference was by Ti. The Ti upper limit in Fig. 3 represents the substrate Ti concentration.

Because of the complex geometry of the crater it was not possible to use conventional correction procedures on the probe data; the results should accordingly be considered as only semi-quantitative. The geometry affects the absolute X-ray flux received by the spectrometer but does not affect strongly the relative intensities from different elements. The



Fig. 2 Bottom of the 110  $\mu$ m crater showing physical evidence for meteoroid residue. Scale bar=10  $\mu$ m.

measured relative abundance ratios should be fairly accurate. This ratio technique has been applied with considerable success<sup>4,5</sup> to the analysis of other specimens with non-planar geometry.

The small crater was analysed with a point beam at several spots around the crater floor. Iron and sulphur were detected as major elements, and Ni and Mg as trace elements. The small size of the crater makes quantitative analysis difficult but the implied Fe:S ratio is roughly consistent with FeS. The Fe, S and Ni residues indicate that the crater may have been produced by a troilite (or pyrrhotite) meteoroid. Troilite is a common mineral in most meteoritic types. The Fe:Mg ratio was 50:1, so the Mg content is not of major concern.

The results of the two analyses on the large crater are shown in Fig. 3. The two runs agree well with each other, and the agreement with carbonaceous chondrites is within a factor of two for the seven elements quantitatively analysed. Sulphur, a cosmically abundant but volatile element, is not plotted because it was not looked for during that analysis. A sulphur analysis done at a later date, however, showed that sulphur is present in the crater with quite high abundance, qualitatively similar to the abundances of the Fe, Mg, Si group, and compatible with chondritic abundances.

The crater analyses indicate that, for two randomly sampled meteoroids, a 9 µm particle had a composition consistent with troilite and a 30 µm particle contained chondritic abundances. Particles of similar size and chemistry are common in carbonaceous chondrite meteorites and the implication is that the source material for interplanetary dust may be similar to this primitive meteoritic type. Evidence for a similarity to carbonaceous chondrites rather than to other meteorite types comes from the inferred grain sizes within the 30  $\mu$ m particle. As no known mineral contains cosmic abundances, the particle must have been composed of an aggregate of smaller mineral grains. For the composition of the particle to be close to cosmic abundances the constituent grains must have been considerably smaller than 30  $\mu$ m. Few chondritic meteorites outside the carbonaceous group have typical grain sizes as small.

Although these may be the first laboratory analyses of interplanetary grains, there are several other lines of evidence that support similarities between chondritic meteorites and interplanetary material. Studies of the extra-lunar component of lunar soils have demonstrated that the bulk of the micrometeoroid mass hitting the lunar surface has trace element abundances identical to C1 chondrites. In addition

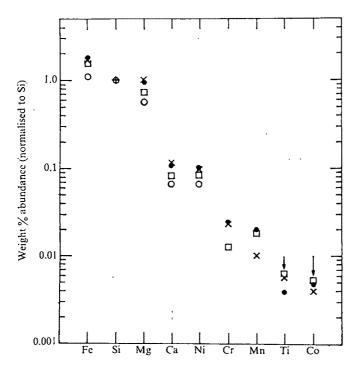


Fig. 3 Elemental abundances in the 110 μm Skylab-IV compared with average carbonaceous chondrite abundances. The open squares and open circles represent different probe runs on the crater. The filled circles and crosses are, respectively, CI and C3 meteorite averages<sup>12</sup>. Ti and Co were not detected in the crater and the upper limits are indicated by arrows.

analyses of meteor spectra1,7 and measurement of mesospheric ion enrichment during meteor showers<sup>8</sup> suggest that the bulk of millmetre-sized particles at 1 AU have abundances close to chondritic values. The apparent similarity between interplanetary material and carbonaceous chondrites should not, however, be construed as evidence that both types of object have a common source. The similarities are probably only a consequence of each type representing primitive, well preserved samples of early solar system materials.

From the microprobe analyses we estimate that some 10% of the two Skylab-IV meteoroids survived as residue. This degree of residue retention is consistent with laboratory simulations9,10 using iron projectiles, but not with studies of micrometeorite craters on lunar rocks11. The difference may arise because the impact of Skylab was on soft metal whereas on lunar rocks it is normally on to silicate materials. The impact conditions are quite different in the two materials, as shown by laboratory observations that for a fixed projectile energy, a hypervelocity crater in metal will have almost ten times the volume of a crater produced in a silicate target10.

The successful detection of residues in the Skylab-IV micrometeoritic craters suggests that if other craters, in appropriate targets, can be recovered from space it may be possible to carry out a statistical evaluation of elemental abundances in debris generated by comets. Primitive meteorites contain large (> 30 µm) inclusions of materials with strongly non-cosmic abundances (such as olivine, magnetite, sulphides, calcium aluminium inclusions and chondrules). Residue analysis of 100 craters or so in the 100-300  $\mu$ m size range would show whether or not these types of particles were also incorporated into the dust-ice matrix of comets. Positive or negative results of such a search would have fundamental implications regarding the interrelationships between comets and meteorite parent

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### Lunar magnetometry and mantle convection

THE hypothesis that the lunar mantle is at present convecting in a manner somewhat similar to that of the Earth is strongly supported by further analysis of lunar magnetometer data. Using the assumption that the electrical conductivity of the lunar interior varies with temperature, as laboratory measurements on rock-forming silicates indicate, I conclude that the Moon has a thermal boundary layer which is no more than 200 km thick and an interior which is relatively isothermal. Such a temperature profile is predicted by the hypothesis of a slowly convecting lunar mantle; it is in serious conflict with the hypothesis that the Moon is statically cooling and has a rigid crust extending to a depth of many hundreds of kilometres.

Early interpretation1 of magnetic transient data from the lunar surface indicated the magnitude of the electrical conductivity of the lunar interior. Sonnett et al.2 argued that these experiments should be interpreted in terms of important compositional differentiation about 200 km below the lunar surface to account for the large electrical conductivity peak there, which the data seemed to imply. Rama Murthy<sup>3</sup> pointed out that such a conductivity profile could result from the presence of an FeS layer at this depth. Kuckes4 concluded that a serious question of uniqueness existed in the Sonett conductivity profile since the data could be fitted equally well with a simple two parameter profile. Subsequent computations by Sonett et al.5 confirmed this conclusion. Hobbs6 applied the Backus-Gilbert test to these two models and argued that the significance of the eight-layer model proposed by Sonett et al. was limited.

Because of the difficulty in assigning a unique conductivity profile to fit the observations, I have chosen to interpret these data in a somewhat different manner, which is much less sensitive to such difficulties. Since the electrical conductivity variation with temperature exhibited by most rock-forming silicates is very strong, direct comparison of temperature profiles with the experimental observations is much more inclined to be unique than are fits of the electrical conductivity profile. Though the exact chemical composition of the lunar interior is not known, the electrical conductivity o can, with reasonable certainty, be assumed to have approximately the exponential variation with temperature T which characterises a semiconductor, namely,  $\sigma = \sigma_0 \exp(-\varepsilon/kT)$  ( $\varepsilon$  an activation energy, k Boltzmann's constant). Measurements of the average lunar density, the lunar moment of inertia and the composition of the lunar material returned to Earth suggest that the composition of most of the lunar interior is relatively uniform. I thus assumed, as a working hypothesis, that the important variation in the lunar electrical conductivity is due to temperature alone and can be written in the exponential form indicated, with the activation energy  $\varepsilon$  and the normalisation constant  $\sigma_0$ fixed though unknown.

To abstract the information needed from the experimental data we continued the practice, initiated by Dyal and Parkin<sup>1</sup>, of comparing in detail the behaviour of transients simultaneously recorded by Explorer 35 and Apollo 12. So that a large amount of data could be considered, individual transients were Fourier analysed in time and the ratio of the dominant amplitude was used to compute experimental values for the field amplification and the lunar transfer function A (ref. 7). The values of A derived by this method conform with those of Sonett et al.<sup>2</sup> in the domain of frequency overlap.

Figure 1 shows rather clearly that only the very outer portion of the Moon can have a significant temperature gradient. A significant temperature gradient extending deeper than about 200 km does not fit the data well.

The effect of varying the temperature gradient below 175 km, that is, below the knee in temperature profile 1, is shown in Fig. 2. The experimental predictions for these three temperature profiles were computed on the basis of the 2.33 eV activation energy reported by Duba *et al.*<sup>8</sup> for olivine. Although all three temperature profiles plotted in Fig. 2 fit the data reasonably

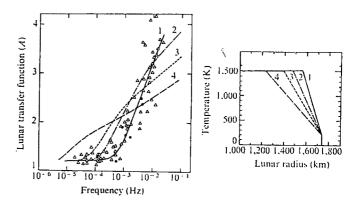


Fig. 1 Experimental values of the lunar transfer function compared to theoretical curves generated by several temperature profiles. The experimental points were obtained by simple Fourier analysis of magnetic transients in the Explorer 35 and Apollo 12 magnetometer records  $\bullet$ , From radial field night-time data;  $\triangle$ , from horizontal field daytime data. The conductivity  $\sigma$  of the outer half of the Moon was assumed to depend on temperature as  $\sigma = \sigma_0 \exp(-\epsilon/kT)$ .  $\epsilon$  was chosen to be 1.5 eV; the general results are not sensitive to the magnitude of  $\epsilon$  if it is greater than about 1 eV. The temperature of 1,500 K for curve 1 was obtained by comparing the magnitude of the conductivity derived to the laboratory measurements of olivine by Duba et al.8. The theoretical curves include a 0.2 contribution to A by the diamagnetism of the solar wind²³.

well, profiles with a temperature difference much greater than 400 K between 1,000 and 1,565 km radius clearly will not. Effectively, the steepness of the experimentally deduced variation of the transfer function A with frequency implies that the electrical conductivity cannot increase by more than an order of magnitude with depth between 1,565 and 1,000 km. Assuming that the significant conductivity dependence in the lunar interior is due to temperature and that the activation energy associated with this temperature dependence is large (as laboratory data indicate) the conclusion that the Moon has a thermal boundary layer which is only a few hundred kilometres or less thick is rather definite.

In the theoretical formulation of the transfer function A, the frequency  $\omega$  and conductivity  $\sigma$  appear only as a product, that is, as ωσ. Thus a change in the value of the conductivity normalisation constant  $\sigma_0$  has the effect of only translating the curves for A horizontally in Figs 1 and 2. The shapes of the theoretical fits shown in Fig. 1 are not particularly sensitive to the value of the activation energy constant ε provided that it lies in the range to make the temperature dependence strong. For rock-forming silicates at the temperatures in question this assumption is fulfilled. The allowable temperature gradient in the interior region shown in Fig. 2 varies inversely with the activation energy  $\varepsilon$ . The vital features, required to obtain the conclusions derived, are that electrical conductivity increases rapidly with temperature, a lunar transfer function A which increases steeply with frequency to a value greater than 3, and the assumption of compositional uniformity.

Leavy and Madden<sup>9</sup> have attempted to fit lunar magnetometer data using the temperature profile proposed by Toksoz *et al.*<sup>10</sup> who considered the thermal history of a hot, cooling Moon. For present-day conditions they obtained a temperature gradient extending to a depth of more than 600 km. Leavy and Madden<sup>9</sup> concluded that consistency with the magnetometer data can only be obtained by assuming that the conductivity of the lunar interior is characterised by a very low activation energy, that is, the conductivity does not change very rapidly with temperature. They point out that this property is difficult to reconcile with the measured properties of the materials generally believed to constitute the lunar interior.

The conclusion that the lunar interior below a depth of about 200 km or less is convecting suggests a striking similarity to terrestrial conditions. The older parts of the Earth's lithosphere are about 100 km thick and have a temperature difference of about 1,300° C across them. About one half of the heat flow observed at most continental sites is derived from the mantle, that is, about  $0.8 \times 10^{-6}$  calorie cm<sup>-2</sup> s<sup>-1</sup> (ref. 11). This value yields an average lithospheric thermal conductivity of about 8×10<sup>-3</sup> calorie cm<sup>-1</sup> °C<sup>-1</sup> and an average specific heat generation for the Earth's interior of  $3.7 \times 10^{-15}$  calorie cm<sup>-3</sup> s<sup>-1</sup>. This value for the thermal conductivity, a 200-km lunar lithosphere, and a temperature difference of 1,200° C across it yields a lunar mantle heat flow of  $0.4 \times 10^{-6}$  calorie cm<sup>-2</sup> s<sup>-1</sup> and an average specific heat generation for the lunar interior of  $7 \times 10^{-15}$  calorie cm<sup>-3</sup> s<sup>-1</sup>. The heat flow computed is about one half that observed12; the specific heating is about double that for the Earth.

It is well known that the Earth's mantle convection induced linear features like mountains, oceanic trenches and island arcs, notably absent on the Moon. I note that the stress on a spherical shell due to loading by a linear feature with a fixed force length and angular width varies approximately as  $r/t^2$ where r is the radius of the shell and t is its thickness. The force length associated with such a feature should vary in proportion to gravity if simple loading by a mountain range is in question, or in proportion to the radius of the body if the viscous drag of the circulating eddy in the lithosphere is involved (assuming constant viscosity). In either case the net stresses trying to break up the lunar crust would be about two orders of magnitude lower than on the Earth.

A great disparity apparently exists between the magnitude of the mantle electrical conductivity of the Moon and the Earth. Because of the complications introduced by the high electrolytic conductivity of the oceans and sediments, the electrical conductivity of the Earth's upper mantle is not well known; it is, however, apparently an order of magnitude greater than that of the Moon<sup>13</sup>. In addition to the very strong temperature effect the electrical conductivity is quite sensitive to composition, particularly changes in the Fe3+ concentration; thus, this disparity is perhaps not too surprising.

Turcotte et al.14 conclude that the critical temperature for mantle convection in the lunar interior is about 1,300° C which closely coincides with the temperature derived. Cassen and Reynolds15 have considered the effect of the temperature dependence on viscosity and have shown that convection can play an important role in the lunar thermal history. The thermal

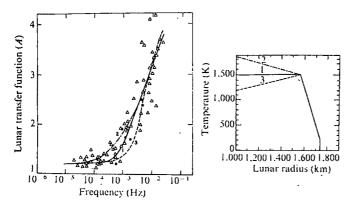


Fig. 2 The variation of the lunar transfer function as the temperature gradient of the lunar interior is varied. From radial field night-time data;  $\triangle$ , from horizontal field daytime data. The electrical conductivity was assumed to vary as  $\sigma = \sigma_z \exp(-\varepsilon/kT)$  with  $\varepsilon = 2.33$  eV. The magnitude of the temperature gradient in the interior region of profiles 2 and 3 is about 6% of that in the thermal boundary layer, that is, outside a radius of 1,560 km.

models of McConnel and Gast<sup>16</sup> which include convection are consistent with our conclusions. Tozer's convective model<sup>17</sup> also predicts a temperature profile in agreement with our analysis; however, the temperature and radioactive heating rate he proposes seem rather low to obtain either convection or the observed value of the observed electrical conductivity.

Without convective transfer of heat most models of the lunar interior lead to the development of a thermal boundary layer too thick to be compatible with my analysis. Toksoz et al.10 have argued for a statically cooling Moon which, at present, has a thermal boundary layer about 600 km thick. Reynolds et al.18 have also shown that an initially cold Moon with a uniform carbonaceous chondritic composition develops a very thick boundary layer.

The computations of Hanks and Anderson<sup>19</sup> show that a present-day thin thermal boundary layer consistent with our conclusion can, however, be generated by the proper combination of surface heat sources and initial temperature conditions at the time of lunar formation. Using a steady state approximation, their work shows that the assumption of complete early differentiation of the radioactive elements to the lunar surface can lead to a wide variety of thermal boundary layer thicknesses depending on the exact balance between initial temperature, absolute magnitude of the radioactive heating rate and the thickness of the heating layer. In any case, the temperatures they discuss are sufficient to make convection likely.

Urey<sup>20</sup> and Gold<sup>21</sup> have long argued for a cold inactive lunar interior. Although exact calculations have not been made, T. Gold (private communication) has argued that deposition of the surface radioactive material somewhat after cold accretion could lead to general consistency with our results. Urey<sup>20</sup> and Gold<sup>21</sup> have also raised concern about the support of the mascons by the lunar lithosphere. While the magnitude of the gravitational anomalies is, in terms of percentage, quite large compared to the terrestrial phenomena, Kaula<sup>22</sup> has argued that isostatic compensation on the Moon is as good or somewhat better than on the Earth and that a 150-km thick lithosphere is sufficient to support the mascons.

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#### Martian terminator waves

I HAVE suggested that the supersonic motion of the Earth's terminator could generate atmospheric waves1, and Raitt and Clark claim to have discovered these waves in the terrestrial thermosphere<sup>2</sup>. The Martian terminator will also be supersonic in a wide band of latitudes in the Martian upper atmosphere and may be expected to produce waves if the region's radiative response occurs on a shorter time scale than the dynamic response.

In Martian low and middle latitudes the terminator moves with a speed,  $V_T$ , of:

$$V_{\rm T} = 240 \cos \phi \, \text{m s}^{-1}$$

where  $\varphi$  is the geographical latitude. If we assume a pure CO<sub>2</sub> atmosphere then the speed of sound, C, is

$$C = 16.2 T^{\frac{1}{2}} \text{ m s}^{-1}$$

where T is the local atmospheric temperature. Supersonic waves may be generated at the equator in regions where T < 220 K, and at latitude 30° if T < 164 K. If we assume that the minimum temperature in the Martian atmosphere is 140 K at the tropopause<sup>3</sup> then these waves can exist for  $|\phi| < 37^{\circ}$ .

In December 1971 Mariner 9 photographed Mars. There were 10 distinct observations of atmospheric wave structure on or near the terminator between latitudes  $+40^{\circ}$  and  $-20^{\circ}$ . Typical wavelengths were 40 km and the lack of any wave structure when photographed through orange filters indicates that the waves occurred in the atmospheric layer above the dust storms that were blowing during the Mariner expedition4. As the dust existed up to 10 km altitude it seems highly reasonable to assume that the terminator waves were generated in the stratosphere (that is, above 15 km) when the stratospheric temperature was sufficiently low. The observed latitudinal extent of the waves agrees well with this hypothesis. There is good reason to believe that the Martian stratosphere is isothermal over a height range of at least 15 km (ref. 3). Provided that the stratospheric temperature is low enough to generate supersonic waves then the resulting disturbances are in phase over that height range. The oscillation produced will, therefore, be evanescent, or very nearly so.

The only waves that will escape destructive interference are those for which the trace of the phase velocity in the direction parallel to the Equator equals the speed of the terminator within the source region of the wave. Only two types of atmospheric oscillations can have supersonic phase velocities. First, very low frequency infrasonic waves which satisfy the dispersion relationship

$$k^{2} = \frac{\omega^{2}}{C^{2}} \frac{\omega^{2} - \omega_{an}^{2}}{\omega^{2} - \omega_{B}^{2}}$$
 (1)

when they are evanescent; and second, the characteristic

surface wave<sup>6</sup>, which is always evanescent, and obeys the relationship

$$\omega^2 = C \omega_{\rm B} k \tag{2}$$

where  $\omega$  and k are the angular frequency of the wave and wavenumber, respectively,  $\omega_B$  is the local Vaisala-Brunt frequency, and  $\omega_{an}$  is the non-isothermal acoustic cutoff frequency, given in terms of the temperature gradient α as<sup>5</sup>

$$\omega_{an}^2 = \frac{\gamma^2 g^2}{4C^2} \div \frac{\gamma g}{2T} \alpha$$

where  $\gamma$  (=1.4) is the ratio of specific heats and g(=3.76 m s<sup>-2</sup>) is gravitational acceleration.

The type of oscillation generating Martian terminator waves can be located by finding  $\alpha$  from equations (1) and (2). For example, the waves at  $+40^{\circ}$  latitude occur when  $V_T = C$ ; thus, the wave parameters are given by  $V_T = C = \omega/(k \cos \theta)$ , where 0 is the solar declination. If we assume  $\theta = 0^{\circ}$ , then  $\alpha =$ 4.26 K km<sup>-1</sup> from equation (1) and 25.6 K km<sup>-1</sup> from equation (2). The first of these is the only realistic value.

It is worth studying Revolution 17 of Mariner 9. Terminator waves were observed at -20° latitude, 20°W longitude. Temperature profiles obtained during this revolution7 indicate that at this latitude the terminator was supersonic from an altitude of 30 km to at least 50 km. The minimum temperature of the tropopause at 38 km is 160 K, and it rises immediately above the tropopause with a gradient of 4 K km<sup>-1</sup>. These experimental results certainly agree very closely with this theory.

There remains, however, one unusual feature of the Martian terminator wave observations. The tilt of the wavefronts corresponds to that expected during the Northern Hemispheric summer, whereas the latitudinal extent of the wave observations seems to indicate that the Northern Hemispheric stratosphere was at a lower temperature than the southern stratosphere.

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## Effects of meridional winds on the interpretation of satellite inclination data

DURING the past decade, the analysis of long term changes in inclination of Earth-orbiting satellites has been used to determine characteristics of the zonal wind field in the Earth's upper atmosphere. As reviewed by King-Hele<sup>1</sup>, results of analyses of this type indicate that, on average, the atmosphere between 200 and 350 km rotates faster than the Earth. Average eastward wind speeds of the order of 100 m s<sup>-1</sup> with respect to the Earth's surface are indicated in the altitude region around

The effect of thermospheric winds on satellite inclination is quite small, of the order of 0.1° over a period of several months; as a result long and accurate observations are required to produce useful information. The deduced wind speed applies to a height slightly above perigee and is most heavily weighted by the low latitude wind field, as the effect is strongest at the equator. Since at least several months of data are required for an adequate analysis, the inferred wind is an average over the local times and seasons covered by satellite perigee during that period.

Recent accurate satellite observations using Hewitt cameras have allowed shorter term changes in orbital inclination to be usefully studied. In this way, local time variations of upper atmospheric winds have been determined. Using data of this type for the orbit of Cosmos 316, King-Hele<sup>2</sup> tentatively inferred an 8-h oscillation n the zonal wind field at altitudes of 150–170 km. Here we show that such an interpretation of the data is not unique. In fact, we suggest that short term variations of the inclination may be more realistically explained in terms of meridional wind effects.

The zonal component of the upper atmospheric wind field is not the only component which affects satellite inclination. The meridional wind field can also have a notable effect, especially if its magnitude is as large as that of the zonal wind. King-Hele<sup>3</sup> treated the effect of a meridional wind on satellite orbits and derived the following approximate relationship between the inclination i and the orbital period in days  $T_{1i}$ :

$$\frac{\mathrm{d}i}{\mathrm{d}T_{\mathrm{D}}} = \frac{1}{3\sqrt{F}} \left[ \Lambda \sin i \left\{ (1-4e)\cos^2 \omega - \frac{1}{z}\cos 2\omega \right\} - \left[ -\epsilon(1-\kappa)^{1/2} \left\{ (1-\frac{1}{4}\kappa) \left(1-\frac{1}{2z}\right)\cos \omega - \frac{1}{4}\kappa \cos 3\omega \right\} \right], \quad (1)$$

where  $\sqrt{F=1-\Lambda T_0}$ , cosi;  $\Lambda$ =atmospheric eastward angular velocity in units of the Earth rotation rate; e=orbital eccentricity;  $\omega$ =argument of perigee; z=ae/H; a=semi-major axis of the orbit; H=atmospheric density scale height at perigee;  $\varepsilon$ =atmospheric northward angular velocity in units of Earth rotation rate;  $\kappa$ =sin² $i/(2-\sin^2i)$ .

This relationship is based on a number of reasonable approximations, and is valid for  $z \gtrsim 5$ . As pointed out by King-Hele, the zonal velocity (parameterised by  $\Lambda$ ) leads to a secular change in i as the value of  $\omega$  of the satellite orbit changes. The meridional velocity (parameterised by  $\varepsilon$ ) has no net effect if  $\varepsilon$  is a constant and if the satellite orbit makes a complete cycle in  $\omega$ . The first condition is probably not valid in the thermosphere, however, and the second is not satisfied when short term changes in inclination are studied.

Recent observations of the meridional wind field using the ionospheric radar backscatter technique (see, for example, Evans<sup>4</sup>) indicate that meridional winds can attain speeds of the order of 100 m s<sup>-1</sup> and that there is a strong dependence on local time and season. Theoretical studies of thermospheric dynamics<sup>5</sup> show an additional dependence on latitude. Since perigee latitude and local time and the season vary over the span of orbital data used in inclination analyses, so does the meridional wind ε. Thus, it seems probable that a non-negligible contribution to the change in a satellite's orbital inclination could result from interaction with the meridional wind field whether or not the satellite perigee completes a cycle in ω.

Perhaps even more relevant to the study of short term inclination changes is the observation that meridional wind effects generally will not average out when  $\omega$  goes through only a fraction of a cycle. Although this is immediately evident from equation (1), we have, for purposes of illustration, integrated this equation for a nominal satellite orbit using various combinations of constant values of  $\varepsilon$  and  $\Lambda$ . The orbit used had the following initial values: a=7,682 km, e=0.15,  $i=50^{\circ}$ ,  $\omega=100^{\circ}$ . The initial perigee height for this orbit was 164 km and the parameter z was about 50. The atmospheric density was assumed to vary only with altitude (no latitudinal, seasonal or local time variation) and the vehicle drag parameter  $(C_DA/W)$  was taken to be 0.02 cm<sup>2</sup> g<sup>-1</sup>. The

time history of the orbit over a 5-month period was established using an orbit simulation computer program available at The Aerospace Corporation. It was found that the relevant orbital variables are described with accuracy sufficient for our purposes by the expressions:

$$\omega = 100^{\circ} + 3t$$
  
 $e = 0.15 - 0.00015t$   
 $T_{10} = (111.5 - 0.0286t)/1,440$ 

where t is the time (d) from epoch.

Integration of equation (1) using the above expressions yields the results shown in Fig. 1 for four different combinations of  $\Lambda$  and  $\varepsilon$ :  $\Lambda=1.0$ ,  $\varepsilon=0$  (a);  $\Lambda=1.2$ ,  $\varepsilon=0$  (b);  $\Lambda=1.0$ ,  $\varepsilon=0.2$  (c);  $\Lambda=1.0$ ,  $\varepsilon=0.4$  (d). Curves a and b show the monotonic decrease in inclination that results from purely zonal winds, the larger value of  $\Lambda$  leading to a more rapid decrease in i. The slower variation of i near T=110 min and T=108 min occurs when the satellite perigee goes through apex in the Southern and Northern Hemispheres, respectively. Curves c and d show how meridional winds can affect the time change in inclination. After a complete cycle in  $\omega$  (at T=108 min) the net effect of these constant meridional wind fields is seen to cancel out; but over a time period less (or more) than a com-

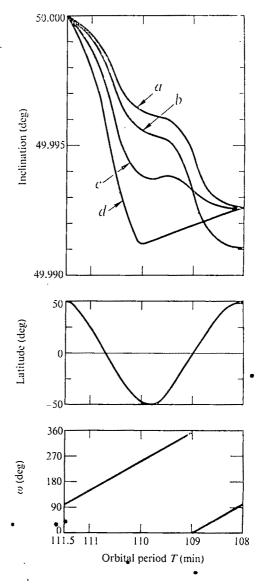


Fig. 1 Orbital inclination, latitude of perigee, and argument of perigee as functions of orbital period. The zonal and meridional wind parameters are:  $\Lambda=1.0$ ,  $\epsilon=0$  (a);  $\Lambda=1.2$ ,  $\epsilon=0$  (b);  $\Lambda=1.0$ ,  $\epsilon=0.2$  (c);  $\Lambda=1.0$ ,  $\epsilon=0.4$  (d).

plete cycle, the change in inclination is substantially affected by the meridional component. The rate of decrease in inclination, attributable to the prevailing zonal component, is modified by the meridional component to be faster or slower depending on whether the satellite motion is against or with the meridional wind. Thus, for example, if the earlier portions (up to T=110min) of curves c or d were used to deduce zonal winds assuming no meridional wind effects, the inferred value of  $\Lambda$  would be larger than actually so. It is seen in curve d that if  $\varepsilon$  is large enough, the inclination can actually increase when the satellite moves in the same direction as the meridional wind. In fact, the inclination data for Cosmos 316 presented by King-Hele<sup>2</sup> show periods during which the inclination increases in a manner quite similar to that depicted in Fig. 1. In an analysis based on only zonal wind considerations, such data would be difficult to fit (as King-Hele acknowledged) and the best fit attainable would in essence disregard such features.

We emphasise that the curves presented here are only illustrative. As pointed out previously, the meridional wind field depends strongly on season, local time and latitude; therefore, in realistic situations the effect of meridional winds would be highly variable depending on the orientation of the satellite orbit in space. The meridional wind effects shown in Fig. 1 are undoubtedly extreme, as we have assumed  $\epsilon$  to be constant and of magnitude comparable to peak values rather than zonally averaged values. Nevertheless, even with smaller values of  $\epsilon$ the effect of meridional winds can easily modify the rate of change in inclination attributable to pure zonal winds. Thus, it seems that inclination data, particularly those encompassing less than a cycle in ω, cannot safely be interpreted on the basis of only zonal wind behaviour.

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# Relativistic angular momentum of power circulation

THE purpose of this letter is to show that a special relativistic turning effect results from the angular momentum of the mass circulation connected with closed circuit mechanical or electromagnetic power transmission.

As a result of the relativistic equivalence of energy and mass, the momentum density of a body or a volume section through which power is transmitted is a function of the power flowing through it. In terms of relativity, this follows from the symmetry of the energy-momentum tensor<sup>1</sup>. If P<sub>i</sub> is the power flow per surface unit and c the velocity of light, the relativistic momentum density of the power flow is

$$\mathbf{g} = \mathbf{P}/c^2 \tag{1}$$

The direction of g is the same as that of the power flow, and P can be attributed to any kind of energy transmission, such as mechanical, thermal or electromagnetic. In the latter case, P is identical with the Poynting vector  $\mathbf{P} = \mathbf{E} \times \mathbf{H}$  ( $\mathbf{E} = \text{electric}$ field strength, H = magnetic field strength). For example, the relativistic momentum of a rotating line shaft or of an electric cable of length I transmitting power N is

$$G = NI/c^2 \tag{2}$$

In other words, a body transmitting power can incorporate a momentum without moving in the direction of the momentum.

Of particular interest is the case in which power (that is, mass) circulates on a closed path within a body or a space section. A simple zero-loss power circuit may consist of an electric and a mechanical section: a loss-free electric motor drives a loss-free generator by a line shaft and the electric power delivered by the generator is transmitted back to the electric motor by a loss-free electric cable, so that the power circulates continuously between the electric motor and the generator. Each section of such a power circuit contains a momentum given by equation (2).

A closed mechanical power circuit can be obtained by connecting the ends of a flexible transmission shaft such that it forms a closed ring with mean radius R and that a torque M is conserved in the whole shaft. If the shaft is in rotation about its circular axis with angular velocity  $\omega$ , a power flow  $N=M\omega$  (a mass flow  $m=N/c^2$ ) circulates through it. Equation (2) then yields the angular momentum of this power circulation:

$$J_1 = 2\pi R^2 M\omega/c^2 \tag{3}$$

Inother words, the annular shaft posseses an angular momentum about the axis perpendicular to its plane (a axis) without rotating about this axis. If the shaft rotates freely about the a axis with an angular velocity  $\Omega$ , then the total angular momentum about the a axis is  $J = J_1 + \theta_1 \Omega$ , where  $\theta_1$  is the moment of inertia of the annular shaft about the a axis. Because of the law of conservation of angular momentum, a decrease of  $J_t$ on account of a decreasing power circulation results in a corresponding increase of  $\theta_1\Omega$ . Suppose that  $\omega$  slows down at a rate  $d\omega/dt$  because of internal friction of the flexible shaft. Then the relation between the angular acceleration  $d\Omega/dt$  about the a axis and the rate of decrease of power circulation  $M d\omega/dt$  is

$$d\Omega/dt = -(1/\theta_1) dJ_1/dt_1$$

$$= (-2\pi R^2 M/\theta_1 c^2) d\omega/dt$$
(4)

Electromagnetic power circulation can easily be achieved in a closed superconducting circuit. Figure 1 resembles such a circuit and consists of a toroidal capacitor with a potential difference U between the inner and the outer toroid. Through the

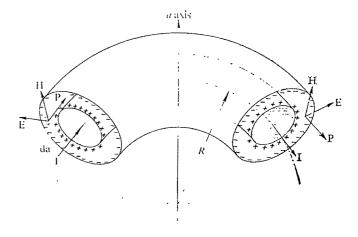


Fig. 1 Illustration of the power circulation in a toroidal capacitor,  $P=E\times H$  - Poynting vector; E= electric field strength; H= magnetic field strength; I= current through the inner superconducting toroid; da - area element of the cross section.

inner superconducting toroid a current I is flowing. At each point of the free space between the toroids there exists a magnetic field  $\mathbf{H}$  and an electric field  $\mathbf{E}$ . The electromagnetic power flow within the toroids is given by

$$N = \int_{1}^{2} \mathbf{P} \, \mathrm{d}\mathbf{a} = UI \tag{5}$$

where P is the Poynting vector and da is an area element of the cross section 1-2 perpendicular to P. Equation (1) yields the momentum density of the electromagnetic field, and the angular momentum of the electromagnetic power circulation about the principal axis of the toroids (a axis) is given by

$$J_2 = (2\pi R^2 U I)/c^2 \tag{6}$$

where R is the mean radius of the toroids.

As the total angular momentum of the toroidal capacitor must be conserved, a change of the electromagnetic power circulation results in a transfer of angular momentum from the electromagnetic field, equation (6), to the conductors. Suppose that the electromagnetic power circulation changes at the rate dN/dt = UdI/dt + IdU/dt, then the corresponding angular acceleration of the toroids about the a axis is

$$\frac{\mathrm{d}\Omega}{\mathrm{d}t} = \frac{2\pi R^2}{\theta_2} \left[ \frac{I \, \mathrm{d}U}{c^2 \, \mathrm{d}t} + \left( \frac{U}{c^2} - \frac{m_e}{e} \right) \frac{\mathrm{d}I}{\mathrm{d}t} \right] \tag{7}$$

with  $e/m_e$  the specific charge of the conduction electrons and  $\theta_2$  the moment of inertia of the toroids about the a axis.

A change of the electromagnetic power circulation can most easily be realised either by a change in the potential difference or by a change in the current intensity. In a practical laboratory experiment, a reduction of the potential difference U can easily be obtained by an internal discharge of the toroids; alternatively the current I can be reduced by heating the superconducting toroid above its critical temperature. Superconducting cables at present under development are designed for power capacities of up to 10 GW (equivalent to mass flow rates of 0.1 mg s<sup>-1</sup>). Under laboratory conditions, much larger power capacities are possible<sup>2,3</sup>, Suppose that a power of 10 GW circulates within a closed circuit cable with R=3 m. Equation (6) yields a relativistic angular momentum of 64 g cm<sup>2</sup> s<sup>-1</sup>, which should be measurable by present means.

The most intense power circulation achieved so far is in high power ring laser (such as the  $CO_2$  laser at Los Alamos<sup>4</sup>). For example, a laser pulse of  $10^4$  J circulating in a path with R=3 m has an angular momentum of  $10^3$  g cm<sup>2</sup> s<sup>-1</sup>.

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# Plate tectonic origin for the Cape Fold Belt?

SEVERAL theories based on geological findings have been proposed for the origin of the Cape Fold Belt<sup>1-3</sup>. An understanding of the tectonic conditions leading to the folding is not only of academic importance, but may be valuable in assessing the possibilities of finding oil in the southern Karroo.

Newton<sup>1</sup> argues that the Cape Fold Belt does not resemble a typical 'plate tectonics' orogen in respect of structural style,

sedimentation or igneous and metamorphic activity. A gravityfolding model controlled by the structure of the pre-Cape rocks is proposed. De Swardt et al.2 suggest that the Cape orogeny is of Alpine type, generally ascribed to gravity sliding away from a median zone of excessive uplift, with movement of the cover over a more rigid basement, which is accompanied by folding and thrusting. It is further pointed out that the best explanation for orogenies of Alpine type is that of collision of crustal plates. Sharp<sup>3</sup> implies that diamond-bearing kimberlites are the result of magmatic processes at the deep end of a subducted oceanic plate. The emplacement should occur deep beneath continents and the kimberlite intrusions parallel to fold mountain ranges. South African fields are stated as a specific example of the suggested process. Before the breakup of Gondwanaland, an oceanic plate was underriding the continent; the surface collision produced Cordilleran type mountains, the present Cape Fold ranges. It is shown that the diamond fields occur in bands parallel to the Cape Fold Belt. In spite of the fact that several prominent geophysical anomalies exist in the area, these models were exclusively based on geological arguments. We feel that useful reconstruction of the tectonic history must take the available geophysical evidence into consideration.

An array of three-component magnetometers recorded magnetic variation fields in 1971 over a large part of South Africa<sup>5</sup>. The principal result was the discovery of a linear body of highly-conductive material under the west central part of the Cape Fold Belt (Fig. 1). Although this structure passes south of the

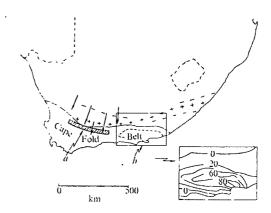


Fig. 1 Magnetic, gravity and conductivity anomalies in the southern point of Africa in relation to the Cape Fold Belt. The maximum (+) and minima (-) of the Beattie magnetic anomalies are indicated. The gravity anomalies are departures from an Airy model of isostatic compensation with crustal thickness 30 km. The inset map is adapted from ref. 6 and the gravity units are in mgal. The arrows represent the negative of the in-phase induction vectors from transfer functions calculated from the estimated normal horizontal components to the anomalous vertical variation field. These vectors that point towards a conductive body are shown for a period of 128 min for the five stations nearest to the anomaly. The approximate position of the conductive Structure is indicated. a, Conductivity anomaly; b, gravity anomaly.

southern corner of the triangular array it could be established that a large highly-conductive body elongated east—west underlies the Cape Fold Belt. Available information indicates that the conductor is in the lower crust or upper mantle. Further work is under way to define the width and axial line of the anomalous zone.

A large negative isostatic gravity anomaly exists in south-eastern. South Africa at the eastern end of the Cape Fold Belt (Fig. 1). It reaches a minimum of -80 mgal of which about 20 mgal can be ascribed to Cretaceous sediments and is elongated along an east-west axis. This anomaly was originally explained as being caused by a relict root or thickening of the crust which had provided Airy-type compensation for a mountain range since eroded away. It was pointed out that a mountain range which would just be supported by the root

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would be 30 km wide and 1.4 km high. As large stresses are necessarily associated with isostatic anomalies of this large magnitude and area, a sequence of failures was predicted by Hales and Gough<sup>6</sup>, leading finally to uplift near the anomaly axis and reduction of the isostatic anomaly and the stresses. There are no large scale isostatic anomalies in the western half of the Cape Fold Belt which could be explained by postulating that the process of uplift is much more advanced there than in the east. Large vertical movement had in fact been observed in the western region<sup>8</sup>.

To explain the conductivity and gravity anomalies, Gough9 suggested that the isostatic anomaly might be the gravitational signature of an east-west trending linear plume in the upper mantle which also produces the conductivity anomaly under the west-central Cape Fold Belt.

The most prominent linear geophysical feature in South Africa is the Beattie Ridge magnetic anomaly10 which lies to the north of and, for most of its 900 km length, parallel to the Cape Fold Belt (Fig. 1). The anomaly consists of a maximum with amplitude ranging from about 100-500 ntesla (total intensity) flanked over large distances by long linear minima. No associated gravity anomaly exists. Deep geo-electrical soundings (J.H. de B., J.S.V. van Z., and F.K.B., unpublished) show that the body causing the anomaly is in the basement beneath the Karroo sediments. Model studies indicate that it is most likely in the middle or lower crust.

This magnetic anomaly cannot be explained in terms of the linear plume hypothesis. On the basis of the available geophysical evidence it is suggested that an oceanic plate underthrust Gondwanaland along one of its margins which coincided approximately with the present southern limit of Africa. Finally a continent-continent collision occurred, producing mountain building which lasted from the Permian to middle Triassic<sup>11</sup>. The most obvious result of this collision is the Cape Fold mountains of South Africa. Overfolding to the north is a prominent feature in these mountains, indicating that the pressure was directed mainly from the south11.

Long linear magnetic anomalies are rarely observed on continents but are typical features in oceanic crust. The long linear Beattie magnetic anomalies may well be caused by the presence of large oceanic slabs thrusted into the original continental edge during or after the formation of a subduction zone near the continental margin<sup>12</sup>.

The roots of collision mountain belts are sialic4 which explains the negative isostatic gravity anomaly associated with the Cape Fold Belt. The absence of an isostatic anomaly in the western half of the fold ranges can still be explained in terms of sub-

Conductivity anomalies in the crust can be related to temperature<sup>13</sup> or composition. Both types of conductivity anomalies are possible in the case of the Cape Fold Belt electromagnetic induction anomaly. If the conductivity anomaly is related to subduction of an oceanic plate and the eventual continental collision, it is most probably caused by a compositional effect. If it is connected to tectonic events surrounding the breakup of Gondwanaland and subsequent tectonic activity, however, there is a strong possibility that the anomaly is associated with anomalously high temperatures and partial melting and that the ridge is in fact a linear region of ascending mantle material as suggested by Gough<sup>9</sup>. This ambiguity as to the cause of the conductivity anomaly can be resolved by a study of the geothermal flux and seismic velocities in the area. This crucial information is not yet available.

Although some features remain unexplained, the geophysical evidence is consistent with the hypothesis that a continentcontinent collision caused the formation of the Cape Fold Belt.

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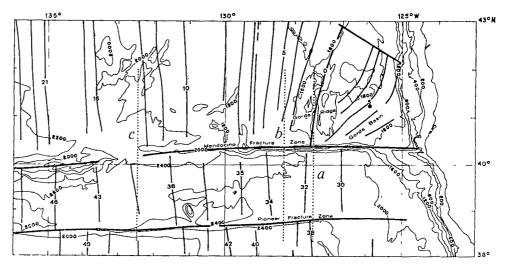
#### Thickness of lithosphere deduced from gravity edge effects across the Mendocino Fault

THE evolution of a lithospheric plate, as it migrates away from the accreting boundary (mid-ocean ridge crest), is mostly a result of vertical cooling by conduction. As density is a function of temperature and pressure, the density structure should be a function of the age of the plate and, in order to preserve isostatic equilibrium, the seafloor should subside as the plate cools1. Thus, the variation of heat flow, seafloor depth and the gravity field are different expressions of the same process, progressive cooling, occurring over the whole thickness of the plate. Sclater and Francheteau<sup>2</sup> have verified these properties through an analysis of the variation of heat flow and depth with the age of the plate. Their model assumed that the plate remains a constant thickness and is floating in hydrostatic equilibrium over the asthenosphere. This led to an estimate of 75 km for the thickness of the plate.

Unfortunately, analysis of variation of the gravity field gives little information about structures in isostatic equilibrium with small lateral variations. Beyond the fact that the underlying volumes are of equal weight, the only information it gives comes from the influence of the lateral variation of density structure on the surface gravity field. As the evolution of the plate is slow, with a thermal constant of about 80 Myr (ref. 3), the lateral variations are small and difficult to detect, except perhaps near the accreting plate boundary. Talwani et al.5 have shown, however, that over the Mid-Atlantic Ridge, gravity data combined with crustal seismic refraction data6 imply the existence of a low density upper mantle body of so-called 'anomalous mantle' at shallow depth (less than 40 km) below the surface. Their conclusion is in qualitative agreement with the low densities predicted by the plate tectonics model. But Morgan7 has argued that gravity data alone cannot rule out the existence of a much deeper compensating body. Keen and Tramontini<sup>8</sup> have assumed that this compensation comes from a mass with a uniformly low density contrast, extending to depths of 200 km.

This controversy could be settled<sup>3</sup> with gravity data alone, by studying active or fossil transform fault areas with large offsets, which juxtapose lithospheric plates of different ages (Fig. 1). The resulting large gravity edge effects are quite sensitive to the depths of the compensating masses. We show here that the gravity edge effect produced by the fossil Men-

Fig. 1 Bathymetric map, after Chase et al. 18, showing gravity profiles a, b, and c (see text). Heavy lines, magnetic lineations with ages given in Myr (after Atwater and Menard 11). The Mendocino Fracture Zone juxtaposes sections of lithosphere which are of different ages: across profile a, 0.3 and 31 Myr; b, 3 and 33 Myr; c, 11 and 40 Myr.



docino Fault—a transform fault—can be explained with Sclater and Francheteau's plate model<sup>2</sup>, and that the most probable thickness of the plate is  $75\pm25$  km.

Figure 1 shows the location of three gravity profiles<sup>9,10</sup> across the Mendocino Fracture Zone. Magnetic lineation data<sup>11</sup> show that the lithosphere on each side of the fault differs in age by 30 Myr. Figure 2 shows the free-air gravity anomaly profiles and the corresponding density models proposed<sup>9,10</sup> to explain them. The crustal density structure adopted<sup>9,10</sup> for profiles a and b (Fig. 2) are based on nearby seismic refraction measurements12 using the Nafe and Drake velocity/density relationship<sup>13</sup>. There is no seismic refraction station near profile c(Fig. 1) but Dehlinger et al.9,10 projected results from refraction stations hundreds of kilometres away. In addition, they did not allow for the velocity anisotropy at the Moho interface<sup>14-16</sup>. The density structure they obtained for the mantle is, consequently, rather heterogeneous. In spite of these limitations, their results show the existence, on profiles a and b (Fig. 1), of an edge effect with a 70 mgal low about 100 km wide south of Mendocino. They explain this by invoking a compensating mass which is about 0.2 g cm<sup>-3</sup> lighter in the upper part of the mantle, as originally proposed by Talwani et al.5. The edge effect decreases in amplitude but increases in width on profile c (Fig. 1), as predicted by the plate tectonics hypothesis of thermal evolution of the lithosphere.

We concentrate on profiles a and b (Fig. 1) which have a better crustal seismic refraction control and in which the contrast in thermal structure should be greatest, according to the plate tectonics model. The crustal density structure adopted was given by Dehlinger et al.<sup>9,10</sup>. The density structure of the mantle is deduced from the vertical temperature distribution computed using Sclater and Francheteau's model<sup>2</sup> for a lithospheric plate of corresponding age. We also use their constants. The temperature of the asthenosphere is taken as 1,300° C and the density of mantle material at 0° C as 3.4 g cm<sup>-3</sup>. The only variable parameter is the thickness of the lithosphere, which we choose to vary between 25 and 200 km.

In practice, the density distribution within the lithosphere is computed by assuming that the ocean floor is the top of the lithosphere, but for the part of the lithosphere corresponding to the crust we adopt the density distribution deduced from seismic refraction results. We divide the remaining part of the lithosphere into layers of constant density differing by 0.01 g cm<sup>-3</sup>; the mean density of the layer is obtained from the Sclater and Francheteau model<sup>2</sup>. Corresponding columns on each side of the fracture zone do not have exactly the same weight, because the temperature has been computed for a constant volume lithosphere and because the crust is ignored in the temperature–density model. We restore hydrostatic equilibrium between the two columns by applying to the older a small thickness correction which is proportional to the difference

between the mean computed density of a given layer and the density of the asthenosphere. We also ignore the modification of temperature structure which occurs because of heat flowing by conduction across the fossil transform fault. It can be shown that both simplifications introduce only second order modifica-

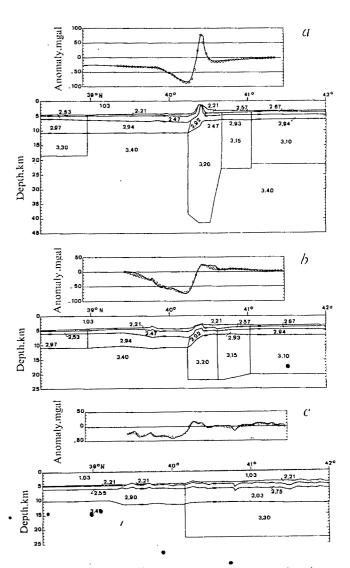


Fig. 2 Gravity profiles (free-air anomalies), and crustal sections along profiles a (along 127° 20′W), b (along 128° 20′W) and c (along 132° 30′W). In crustal sections, depth is shown from sea level, and densities (g cm<sup>-3</sup>) are given. Data from refs 9 and 10.

tions. We felt that the many uncertainties in the data did not warrant testing more elaborate models, such as that proposed by Parker and Oldenburg<sup>17</sup>. Figure 3 shows the density models for profiles a and b.

Figure 3 shows the difference between the observed anomaly and computed gravity anomaly for different models in which the only parameter changed is the thickness of the lithosphere. If the plate tectonics model is correct and if the thickness of lithosphere adopted corresponds to reality, the resulting difference would be zero everywhere. If the thickness is too large then the edge effect is too large and the difference should be positive south of Mendocino and negative north of it. Comparison between computed and observed gravity anomalies shows that the best fits are obtained for a thickness of 75 km (Figs 3 and 4). The data are not good enough to exclude definitely a thickness as large as 100 km or as small as 50 km, but a thickness of 25 km is definitely too small and a thickness of 200 km too large. Thus, models of the type proposed by Morgan<sup>7</sup> or Keen and Tramontini<sup>8</sup> are not compatible with the data.

A thickness of 75 km also fits profile c where the crust is about 10 Myr older than it is near a and b (Fig. 4c). In the absence of nearby seismic refraction stations, we assumed that the crustal structures correspond to those given as a function of age by Goslin  $et\ al.^{18}$ . In addition, the temperature structure may already be affected significantly by the conductive flow of heat through the fracture zone. Consequently, profile c is not as significant as the others.

The lithospheric thickness of 75 km obtained here is identical to that obtained by Sclater and Francheteau<sup>2</sup> on the basis of different types of data (heat flow and depth). This confirms that such a plate tectonics model is successful in accounting for the main physical characteristics of the evolution of a lithospheric plate in its first 40 Myr or so. It also considerably strengthens the choice of physical parameters. The thickness is actually an estimate for a plate 30–40 Myr old. The tempera-

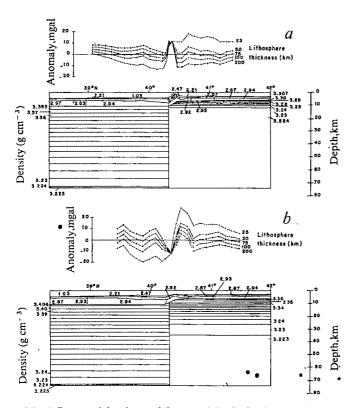


Fig. 3 Proposed density models across Mendocino Fracture Zone along profiles a and b. The thickness of the lithosphere chosen for the models is 75 km. The anomaly curves show the difference between observed and computed gravity anomalies for different lithosphere thicknesses. The density layers have been extended to infinity at both ends to avoid artificial edge effects.

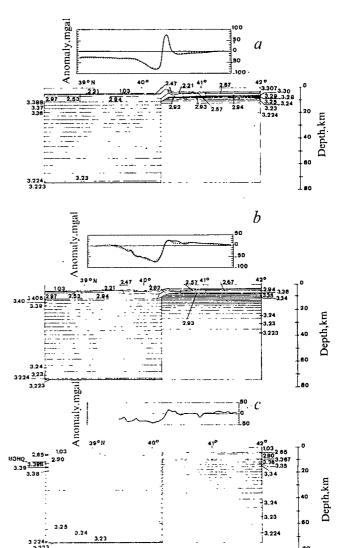


Fig. 4 Proposed density models across Mendocino Fracture Zone along profiles a, b and c and the corresponding observed (solid lines) and computed (dotted lines) gravity anomalies. The thicknesses of the lithosphere chosen for the models is 75 km. For c, the density model of the crust is a function of the age<sup>19</sup> (see text).

ture structure at the accreting plate boundary does not depend on the thickness of the plate; that is controlled by the vertical convection term and not by the conduction term. Thus, this model can be compared with that proposed by Parker and Oldenburg<sup>17</sup> in which the thickness, Z, of the lithosphere changes with age roughly according to the formula:

$$Z(t) = 9.4t^{\frac{1}{2}}$$
 km

where t is time in Myr.

Thus, Z (30-40 Myr)  $\sim$  55 km and the estimate we obtain is in fair agreement with both models within the inherent uncertainties of the data and computations. We feel, however, that more systematic studies, with better seismic refraction and reflection control, across transform faults which juxtapose lithospheric plates of different ages should provide much better constraints to models of lithospheric evolution.

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# Volcanism and glaciation during the past 40 millennia

CLOSE relationships between the timing of volcanic eruption and of glaciation in the past millennia have been noted<sup>1-3</sup> together with the possibility that the three main Neoglacial ice advances were contemporaneous with the three well dated volcanic activity periods in the Southern Andes<sup>3</sup>. Here, I show that glacial advance phases in the Western Northern, Eastern Northern and Southern Hemispheres are mainly synchronous with volcanic activity periods in New Zealand, Japan and southern South America over the past 40,000 yr (the period of reasonably accurate <sup>14</sup>C measurements).

Information on 14C-dated volcanic eruptions and glacial advances is presented in Table 1 together with estimates of their relative magnitudes. All dates used in subsequent discussion are stated in 14C years. The volcanic eruption dates include all those summarised in refs 4, 5 and 6 for New Zealand, ref. 7 for Japan, ref. 1 (after Auer) for southern South America. The glacial advance dates were summarised from a global compilation of 14C-dated advances from many sources. These glacial dates were separated into phases of contiguous advance which were distinct from adjacent phases by at least 200 yr, with the exception of the glacial advance phases during the past two millennia which could be more finely separated. The estimates of magnitude in Table 1 were based on a scale of 1-3 as follows: for the volcanic ash dates, the magnitude of the New Zealand eruptions was determined from the published descriptions<sup>4,6</sup> of the area covered by the

ash showers, with 3 = very widespread, 2 = widespread and fairly widespread and 1 = local. Estimates of the magnitude of Japanese volcanic eruptions were based on the descriptions accompanying the <sup>14</sup>C-dated material<sup>7</sup> as follows: 3 = a major eruption including eruptions which produced a thick pumice layer, 2 = a minor eruption, 1 = a mudflow or (rarely) a lava flow. For the Northern Hemisphere Pleistocene glacial advance phases, a value of 3 indicates a major mid-latitude continental ice advance, a value of 2 a moderate advance and a value of 1 a minor advance. For the subsequent 'Neoglacial' period which occurred after the last readvance of the Wisconsin ice sheet around 7,700–8,300 yr b.p., a value of 2 indicates a major advance of the alpine and polar glaciers and a value of 1 is a minor advance.

The dates for volcanic activity in Table 1 show a tendency for synchronous eruption phases in New Zealand, Japan and southern South America. All eight of the South American volcanic waves are identical with Japanese volcanic phases and six of these are also identical with New Zealand volcanic phases. Of the 18 New Zealand volcanic phases since 33,000 yr b.p., when Japanese dates are first available, 15 are synchronous with Japanese phases, 13 of these by variations of from 0 to less than 300 yr and the other two by variations of 400 and 600 yr. This tendency towards historical synchroneity can also be seen in volcanic eruption dates since 1500 AD (ref. 1) which show that major eruptions occurred in widely separated areas in 1660, 1693, 1694, 1783, 1831, 1846, 1886, 1888 and 1902 AD. Lamb1 has stated that the existence of volcanic synchroneity seems to indicate that stresses build up within the Earth's crust which lead to simultaneous fractures over a wide region.

The dates for glacial advance in Table 1 outline the recognised periods of glacial activity in the late Wisconsin and Holocene. Major continental advances occurred (×1,000 <sup>14</sup>C yr b.p.) around 41.0, 21.0–23.3, 19.4–19.7, 13.7–14.7 and 12.6–13.3, with moderate advances around 35.0–36.9, 30.5–33.5, 17.9–18.6, 16.5–17.2, 10.4–11.0 and 7.7–8.5. Following these large scale glacial advances, there were major Neoglacial advances of alpine and polar glaciers around 4.5–5.3, 2.1–2.9 and 0.04–0.5. The global synchroneity of glacial advance is again evident in the compiled dates in Table 1. The smaller number of Eastern Northern and Southern Hemisphere advance phases could indicate either insufficient knowledge or a genuine lack of glacial activity.

The proximity of the volcanic eruption and glacial advance dates in Table 1 is high. Because of limitations in laboratory technique, no 14C dates are available for Japanese volcanic eruptions before 33,200 yr b.p. and there are no dates before around 17,000 yr b.p. for the southern South American eruption waves. The only earlier information is the New Zealand dates of eruptions around 41,700 $\pm$ 3,500 and 41,000 (67% probability) yr b.p. These dates correspond with the major Gahanna ice advance of the Eire lobe (Fig. 13 of ref. 8). There is no evidence of volcanism during the moderate glacial advance phase around 35,000-37,000 yr b.p. Following this advance phase, all subsequent glacial advances correspond fairly closely with dated volcanic eruptions except the minor advance phase around 15,600-15,700 yr b.p. for which no volcanic dates are available. In two instances there was a lag of 200-300 yr between the volcanic eruption and glacial advance dates: the very minor readvance around 9,200-9,500 yr b.p. which followed a volcanic phase around 9,500-9,800 yr b.p. and the last Wisconsin ice advance around 7,700-8,500 yr b.p. which followed volcanic eruptions around 8,650-8,950 yr b.p. These lags may be climatically significant if they indicate the time necessary for maximum ice advance to occur, once ice build-up has been triggered by volcanic action. Of the eighteen phases of volcanic activity and glacial advance since 17,200 yr b.p., all but four show a lag in ice advance of from 100 to 300 yr following initial volcanic eruption. In three other cases, there is no lag and in one instance, the date of glacial advance precedes volcanic action by 100 yr. Before 17,200 yr b.p. the volcanic and glacial phases are not as well defined; their 14C

Table 1 Periods of glacial advance and volcanic activity since 42,000 <sup>14</sup>C yr b.p.

Glacial advance dates (×1,000 <sup>14</sup> C yr b.p.) Western Eastern				Volcanic activity dates (×1,000 <sup>14</sup> C yr b.p.)				Southern
Northern Hemisphere	Northern Hemisphere	Estimated magnitude	Southern Hemisphere	New Zealand	Estimated magnitude	Japan	Estimated magnitude	South America
~41.0	26.0	3	262.260	41.0-41.7*	3			
35.0–36.0 32.0	36.0 30.5–33.5	2	36.2–36.9	30.1*	2	30.2-33.2*	23	
27.0-28.0	30.5~33.5	1		26.3–27.9	_	26.3-27.9*	2,3 2	
24.6		i		2010 2.17		24.1-24.9*	1	
21.7-23.3	21.0	3				21.4-23.0	3	
19.6	19.7	3	19.4	19.8–20.7	3	19.6-20.8*	3	
∫ 17.9–18.5	18.0-18.4	2	18.6			17.9	i	140 1704
17.0	17.2	2	16.5			16.5–17.3	2	16.0-17.0†
15.6–15.7	15.6	1	(4.1	120 147	2	120		13.5-14.0†
∫ 13.7–14.7 12.6–13.3	13.9 13.2	3	14.1	13.8-14.7	2 2	13.9 12.7-13.1	l 2	13.3-14.01
11.5	11.8–11.9			12.5-13.4	2	12.7-13.1	. J	11.9†
10.4-11.0	10.5–10.7	, ,	10.6-11.0	11.2	2	10.4-10.6	1	10.9†
9.2–9.3	9.2	ī	9.5	9.5-9.8	ī	10.4-10.0	•	1017
£7.7–8.3	7.8-7.9	ż	8.5	8.6-8.8*	ż	8.6	3	8.9
6.5-6.8	6.4-7.2	$\bar{2}$	6.8	7.3	2 2	6.5-7.2	1	
∫ 5.7–6.1		1		6.2	-			
ે 4.7–5.3	4.5-5.0	2	4.5-5.3	5,1-5,2	1,2	4.8	2	4.7–5.4
(3.7	3.8	1	3.7		•_	3.8	Į.	
3.3	3.1-3.3	1		3.4	2	3.4	2	
2.6-2.9	2.6-2.8	2	2.8-2.9	2.8	1,2	2.8	2	2125
2.2-2.4	2.2–2.4	2	2.1-2.5	2.1-2.5	2	2.4–2.5	3	2.1–2.5
1.5-1.8 1.0-1.4		1 1	1.5-1.6 1.0-1.3	1.8-1.9 1.2-1.4	2,3 2	1.3	. 2	
1.0-1.4	0.7	1	0.7-0.8	0.8-0.9	1,2	0.7-0.9	. 4	
0.04-0.5	0.04-0.45	2	0.04-0.5	0.06-0.47	1,2	0.062-0.31	2	0.05-0.45

accuracy is less and there is no consistent pattern of volcanic activity directly preceding glacial advance.

The relative magnitudes of the glacial and volcanic phases show a fairly close correspondence. The major glacial advance around 41,000 yr b.p. was contemporaneous with fairly to very widespread volcanic eruptions in New Zealand. The next major glacial advances occurred around 21,000-23,300 yr b.p. and around 19,400-19,700 yr b.p. Both these glacial phases were contemporaneous with periods of major volcanic eruptions in Japan, and the 19,400-19,700 yr b.p. ice advances occurred at the same time as a very widespread eruption in New Zealand. The last extensive ice advances of the Wisconsin age occurred during the Cary (Earliest Dryas) around 13,700-14,700 yr b.p. and the Port Huron (Older Dryas) advances around 12,600-13,300 yr b.p. The 13,700-14,700 yr b.p. advance phase occurred during moderate to minor volcanic phases in New Zealand and Japan and a major southern South American volcanic wave. The 12,600-13,300 yr b.p. glacial phase exactly corresponded to a period of moderate to major volcanism in New Zealand and Japan. During the Neoglacial, the three major advance phases of alpine and polar glaciers around 4,590-5,260, 2,100-2,940 and 40-460 yr b.p. were exactly contemporaneous with the three major post-Wisconsin volcanic activity phases around 4,700-5,450, 2,150-2,850 and 50-470 yr b.p. in New Zealand, Japan and southern South America as originally noted for the South American data<sup>3</sup>.

The temporal synchroneity of the glacial advance and volcanic activity phases shown in Table 1 may be more than coincidental, if the strong connection between volcanic ash and subsequent glacial advance demonstrated3 since 1500 AD reflects a valid causal relationship. The fact that both the late Wisconsin and Neoglacial ice advances are contemporaneous with major phases of volcanic activity suggests a volcanic trigger for glacial advance. Once this triggering occurs, the subsequent magnitude of glacial advance may depend partly on the magnitude of the volcanic activity and perhaps partly on autocatalytic mechanisms similar to those proposed by Adam9 or Segota10, with the extent of glaciation related to position in the autocatalytic cycle. There is also a possibility that recurrent intervals of diminished solar activity may have created periods of cooler climate<sup>2</sup> which, if coincident with a volcanic activity phase, would have increased the effectiveness of the volcanic ash in stimulating glacial advance.

The close correspondence between periods of volcanic activity and glacial advance since 41,000 yr b.p., shown in Table 1 may have extended throughout the Quaternary. Fuchs and Paterson<sup>11</sup> have reported a similarity between the main epochs of Quaternary glaciation and volcanic activity in a number of regions of low latitude. Hamilton and Seliga<sup>12</sup> found that during the past 100 millennia the temperatures over the Antarctic and Greenland ice sheets were inversely proportional to the amount of volcanic dust which had fallen on these ice sheets.

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<sup>\*</sup> Standard deviation > 1,000 yr. † Indirectly based on <sup>14</sup>C measurements.

#### New dense phases of geophysical significance

THE major increases in density occurring at depths of 400-1,000 km in the Earth's mantle require the formation of phases in which silicon is six-coordinate. Such coordination has been demonstrated in stishovite (SiO2 of rutile type1), in the hollandite form of feldspar compositions2, in the garnet form of (Mg,Fe)SiO<sub>3</sub><sup>3</sup>, in garnet-type MnSiO<sub>3</sub><sup>4</sup>, in perovskite-type CaSiO<sub>3</sub><sup>3</sup> and in SiP<sub>2</sub>O<sub>7</sub><sup>5</sup>—but examples are still few. For the two compositional types believed to provide the bulk of the mantle,  $ASiO_3$  and  $A_2SiO_4$  where A is Mg or Fe, high pressure phase transformations of numerous model compounds such as MnGeO<sub>3</sub>, CaGeO<sub>3</sub>6, Mn<sub>2</sub>GeO<sub>4</sub>, and Ca<sub>2</sub>GeO<sub>4</sub>7 have demonstrated the possibility of high pressure ilmenite, perovskite, strontium plumbate and potassium nickel fluoride forms, respectively, as ultimate high pressure silicate phases; and disproportionation into simple oxides has also been discovered8,9. In the absence of directly observed silicate transformations it is important to delineate the systematic crystal chemistry of each of these structural classes, particularly for those members containing ions closest in size to Fe, Mg and Si, and to obtain further examples of six-coordinate silicon in a range of compounds.

Since the ionic radius of  $Sc^{3+}$  (0.73 Å) is similar to those of  $Mg^{2+}$  and  $Fe^{2+}$  (0.72 and 0.77 Å) while that of  $Al^{3+}$  (0.54 Å) is comparable, with that for octahedral  $Si^{4+}$  (0.40 Å), the composition  $ScAlO_3$  seemed a suitable analogue for perovskite-type (Mg,Fe) $SiO_3$ , although it does not form a compound under normal conditions. We found that an equimolar mixture of amorphous  $Sc_2O_3$  and  $Al_2O_3$  (formed by heating the coprecipitated hydroxides in a vacuum), when reacted in an opposed anvil apparatus at 1,000° C and 120 kbar, gave an orthorhombic perovskite with a=4.933, b=5.226 and c=7.193 Å, isostructural with the rare earth orthoferrites<sup>10</sup>.

Thus an ion of the size of  $Mg^{2+}$  can be accommodated in the A site of the perovskite lattice, while the high pressure behaviour of compositions  $CaTiO_3$ – $CaSiO_3$ <sup>3</sup> have demonstrated that  $Si^{4+}$  can occupy the octahedral B site. It seems highly plausible that  $MgSiO_3$  perovskite will become stable at very high pressures.

Structural refinement based on powder diffraction data showed Sc to be eight-coordinate with an average bond length of 2.25 Å, similar to that for Mg<sup>2+</sup> in pyrope garnet<sup>11</sup>. The Al—O<sub>6</sub> octahedron exhibits normal Al—O bond lengths. We would similarly expect that Si—O<sub>6</sub> octahedra in a perovskite form of MgSiO<sub>3</sub> would conform to the bond lengths found

**Table 1** Predicted\* unit cell volumes (Z = 4) for silicates and other perovskites

Compound	Volume (ų) observed†	Volume (ų) calculated	Sum of formula volumes of constituent oxides‡ (ų)
BaPbO <sub>3</sub> 15	310.8	310.3	334.9
LaScO <sub>3</sub>	266.1	264.1	_
YFeO <sub>3</sub>	224.6	224.5	
CaTiO <sub>3</sub>	223.9	223.5	236.2
SrGeO <sub>3</sub> 16	218.9	220.5	248.1
CaGeO <sub>3</sub>	206.4	206.0	222.0
$MnVO_3$	198.6	199.6	205.8
ScAlO <sub>3</sub>	185.4	185.5	201 §
CaSiO <sub>3</sub>		183.1	204.0
FeSiO <sub>3</sub>	***	165.0	173.9
MgSiO <sub>3</sub>	***************************************	160.9	167.8
$(Mg_{0.86}Fe_{0.14})SiO_3$	***************************************	161.5	168.7

<sup>\*</sup> See text.

Table 2 X-ray structure parameters for pyrochlore type Sc<sub>2</sub>Si<sub>2</sub>O<sub>7</sub> and In<sub>2</sub>Si<sub>2</sub>O<sub>7</sub>

a <sub>0</sub> No. of reflections refined x	Sc <sub>2</sub> Si <sub>2</sub> O <sub>7</sub> 9.287±0.003 Å 11 0.4300±0.0012	In <sub>2</sub> Si <sub>2</sub> O <sub>7</sub> 9.413±0.003 Å 15 0.428±0.0016
$R = \sum (I_{\text{ols}} - I_{\text{edle}}) / \sum I_{\text{ols}} 6 \times \text{Si} - O$	0.0294 1.766±0.007 Å*	0.0235 1.794±0.008 Å*

<sup>\*90%</sup> confidence levels.

in rutile form SiO<sub>2</sub>, or in the pyrochlore silicates discussed below.

Consideration of the volume of the ideal perovskite cell as the product of its unit cell edges has led to a molar volume scheme in which the unit cell volumes of real perovskites are expressed as

$$V = C (A - O)^{x} (B - O)^{3-x}$$

where (A-O) and (B-O) are bond lengths taken from compounds of C-rare earth, rocksalt, rutile or corundum type. For more than 20 known A2+B4+O3 perovskites, including high pressure phases, unit cell volumes can be fitted within  $\pm 0.45\%$  by the values C = 24.29, x = 0.970, and extrapolation with considerable confidence made to the volumes of the geophysically important compositions MgSiO<sub>3</sub>, FeSiO<sub>3</sub> and CaSiO<sub>3</sub> and to solid solutions (Mg,Fe)SiO<sub>3</sub> (Table 1). A<sup>3+</sup>B<sup>3+</sup>O<sub>3</sub> perovskite volumes can similarly be fitted using C = 23.51, x = 1.159. The densities predicted for MgSiO<sub>3</sub> and (Mg<sub>0.86</sub>Fe<sub>0.14</sub>)SiO<sub>3</sub> are very close to those inferred for the zero-pressure densities of the shocked high pressure forms of these phases<sup>12</sup>. Table 1 shows that (Mg,Fe)SiO<sub>3</sub> perovskites would be 4-5% denser than isochemical oxide mixtures (Mg,Fe)O+SiO<sub>2</sub> (stishovite). Consequently, even if disproportionation of MgSiO<sub>3</sub>-FeSiO<sub>3</sub> composition occurs in the transition zone8,9, the oxides are likely to recombine in the deep mantle to form the denser perovskite phase.

In searching for additional examples of six-coordinate silicon we have found that thortveitite, Sc<sub>2</sub>Si<sub>2</sub>O<sub>7</sub>, and its indium isotype, In<sub>2</sub>Si<sub>2</sub>O<sub>7</sub>, are transformed at 1,000° C, 120 kbar to cubic pyrochlore type, with  $a_0 = 9.287$  and 9.143 Å, respectively. In this  $A_2B_2O_7$  structure, A ions are eight-coordinate and B ions six-coordinate. As the only variable atom position in the pyrochlore structure is O(2), at (1/8, 1/8, x), and all six Si—O bonds are equal, the silicate pyrochlores are particularly suited to structural refinement from powder data. The results are summarised in Table 2. An Si—O bond length in Sc<sub>2</sub>Si<sub>2</sub>O<sub>7</sub> of 1.766±0.007 Å was obtained. The Si—O bond length in  $In_2Si_2O_7$  is 1.794  $\pm 0.008$  Å, a value different from that in the scandium compound. This effect may be due to the very marginal ionic radii fit of In and Si in the pyrochlore structure, although similar variations are observed in the polytypes of SiP<sub>2</sub>O<sub>7</sub><sup>5</sup>. The average Si—O bond length for the two silicate pyrochlores (1.780±0.014 Å) is, however, very close to that for rutile-type  $SiO_2$  (1.775±0.006 Å)<sup>13</sup> and a value of 1.78  $\pm 0.01$  Å would seem to define the values expected in high pressure mantle minerals.

Strong evidence has now been obtained (L. Liu, personal communication), by using laser heating of samples pressed between diamond anvils, for the existence of a perovskite phase close to MgSiO<sub>3</sub> in composition and with a unit cell volume almost identical to that predicted in Table 1.

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<sup>†</sup>From references cited in ref. 14 unless indicated otherwise in column 1.

<sup>‡</sup>Rocksalt, rutile or corundum type.

<sup>§</sup>Based on a unit cell volume (348 ų) for Sc<sub>2</sub>O<sub>3</sub> of hypothetical corundum form, by analogy with the C-rare earth and corundum volumes for In<sub>2</sub>O<sub>3</sub>.

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### Tar pollution of Sierra Leone beaches

THE widespread occurrence of pelagic tar and plastic wastes in parts of the Pacific and Atlantic oceans has been described previously. Extensive and considerable fouling of the sandy beaches of Sierra Leone by tar lumps has now been observed at Lumley, Sussex, No. 2, Toke and Mamah villages (Fig. 1) during the past 14 months (June, 1973 to July, 1974). Large quantities of soft, brownish-black lumps (up to 7-8 cm diameter) have been repeatedly washed ashore along the entire stretch (about 6 km) of Lumley beach. This reaches a maximum during June to August, probably because of onshore south-western Monsoonal winds and the increased eastward flow of the Guinea Current during May to October<sup>2</sup>. On August 4, 1974, tar lumps were also observed 1-2 km up the No. 2 River estuary; and during Easter, 1974, a 4-5 km stretch of the sandy beach at Shenge village (7° 56'N 12° 56'W) at the southern tip of Yawri Bay, was also littered with the pollutant. Observations have not been made south of Shenge and north of Freetown (8° 29'N 13° 4'W) but the extent of current pollution tends to suggest that the whole Sierra Leone coastline, and perhaps those of the flanking countries as well, may be subject to frequent tar pollution, probably originating from the heavy, offshore traffic of tankers and ships.

At Lumley beach during ebb tide, tar lumps and other debris are deposited on the sand in a series of crescentshaped aggregations by the receding waves. I have observed ghost crabs (Ocypode spp.) moving along these aggregations and from one crescent to another at different levels of the beach, probably as scavengers. Some workers1 have already noted that the lumps are inhabited by a variety of marine organisms and I have seen several lumps (3-4 cm diameter) bearing colonies of 3-5 small goose barnacles. At succes-

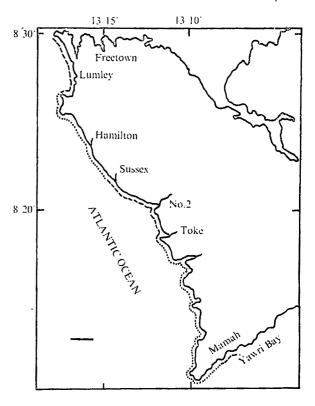


Fig. 1 Western area peninsula of Sierra Leone, showing the sites with tar polluted shores (dashed line) and the probable extent (dotted line) of the peninsular coastline affected by the pollutant. Scale bar represents 2.5 km.

sive spring tides, the lumps and the debris are swept by waves to the supralittoral fringe, leaving the littoral region momentarily clean until the next fresh influx of the pollutant. At the supralittoral fringe, tar lumps are lodged in the soft, dry sand and between the grass roots; here they harden, baked by the hot sun of the dry season (November to April). Fresh tar lumps easily stick to the feet, being effectively removed only by kerosene or local palm oil.

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# In situ methylation of mercury in estuarine sediment

THE methylation of mercury compounds in sediments, believed to be a biological process, has been shown in a number of laboratory experiments1-5. Background levels of methylmercury (CH<sub>2</sub>Hg) have been reported for estuarine sediments from the Mississippi Delta, Mobile Bay and the Florida Everglades. We now report that sediment in San Francisco Bay contained background levels of methylmercury and methylated mercuric chloride (HgCl<sub>2</sub>) in situ.

Methylmercury was analysed using a Varian 1200 chromatograph equipped with an electron capture detector. The column (length 182.8 cm, internal diameter 0.318 cm) contained 5% 'butanidiol succinate' on 'chromosorb W' 80-100 mesh and was maintained at 160° C. The presence of methylmercury was verified by comparing total mercury (Hg) with the amount of methylmercury in the extracts. This verification was carried out on an isotope shift Zeeman effect atomic absorption (IZAA) spectrometer7. Total mercury in sediment samples was also measured on the IZAA spectrometer. Methylmercury was extracted using a modification of the Westöö method8. Ten grams (wet weight) of sediment were placed in a 250 ml glass centrifuge bottle to which 25 ml H<sub>2</sub>O, 10 ml concentrated HCl, 10 g NaCl, 2 ml 5% HgCl<sub>2</sub> (w/v) and 40 ml of benzene were added. The samples were shaken for 15 min and then centrifuged at 490 relative centrifugal force for 8 min.

Thirty millilitres of the benzene fraction were removed and placed in a 60 ml separating funnel. A 1% cysteine acetate solution (6 ml) was added and the samples shaken for 2 min. The cysteine acetate solution was withdrawn, measured and placed in a 30 ml separating funnel to which 3.6 ml 6 N HCl and 4 ml benzene were added. This solution was shaken for 2 min and allowed to stand for 10 min, the benzene was then removed and

Table 1 In situ methylmercury production (ng g-1) by San Francisco Bay sediment

Day 18	
10	28
0.5	. 1.4
17.4	13.6
28.3	0.0*
37.6	7.0
0.1	0.0*
	0.5 17.4 28.3 37.6

<sup>\*</sup> Less than 0.01 ng g-1.

stored for analysis. The extraction efficiency was 92.9% and the limit of detection was approximately 0.01 ng g<sup>-1</sup>. Sediment was analysed for volatile solids9 and particle size distribution10.

To study methylation of HgCl<sub>2</sub> in the natural environment, wire mesh cylinders were embedded in the sediment of an intertidal zone of central San Francisco Bay. Sediment from the inside of each of the four cylinders was removed, separated and weighed; HgCl2, dissolved in deionised water, was added to make 10 and 100 p.p.m. concentrations of Hg (wet weight) in each of two sediment zones (shallow and deep). Mercury was added to only one zone of each cylinder. The shallow zone was formed by replacing the appropriate mercury-sediment mixture only in the upper 1.27 cm of the cylinder. The deep zone was formed by replacing the bottom 6.35 cm with the mercurysediment mixture and covering the upper zone with the original 1.27 cm of sediment. Duplicate 10 g sediment samples were taken from the cylinder on days 0, 18, and 28 of the experiment. Control samples were taken from areas adjacent to the experimental site.

The sediment which represented shallow and deep samples had an average volatile solids value of 2.5%. The particle size distribution was 55.0% medium and fine grained sand, 35.3% silt and 10.7% clay.

Methylation occurred in excess of background levels, being greatest in the deep zones. Background levels of methylmercury fluctuated over the study period from 0.01 to 0.14 p.p.b. CH<sub>3</sub>Hg. We found that the fluctuations in total mercury as well as methylmercury concentration in San Francisco Bay sediment were a common occurrence throughout the year. Total mercury measurements during the study show a continuous decrease from the original values of 10 or 100 p.p.m. Hg. By the 28th day the initial 10 and 100 p.p.m. of Hg was reduced to 0.2 and 0.1 p.p.m., respectively, in the shallow sediment and 2.8 and 10.5 p.p.m., respectively, in the deep sediment. The in situ sediment had a total Hg content of 0.19 p.p.m.

Methylmercury was therefore produced in situ by San Francisco Bay sediments and natural background methylmercury was also observed. The amount of CH<sub>3</sub>Hg produced in situ was far below that found under laboratory conditions1-3 but significantly above background levels. The decrease in the amount of methylmercury found in most cases on day 28 could reflect a decrease in methylation resulting from a decrease in the amount of total mercury present by the diffusion of both methylmercury and inorganic mercury out of the experiment sediments. The loss of mercury was possibly aided by the degradation of methylmercury to its inorganic form, a phenomenon which has been shown to occur under laboratory conditions<sup>3,11-13</sup>.

Andren and Harriss<sup>6</sup> found that background levels of CH<sub>3</sub>Hg decreased with sediment depth (sediment taken from Mobile Bay). This study has shown that at least in the initial phases of methylation, sediment at 6 cm produced more CH<sub>3</sub>Hg than sediment at the surface.

The higher values of CH<sub>3</sub>Hg in deep sediments could indicate: that more methylation occurs under anaerobic conditions; there is a greater loss of CH<sub>3</sub>Hg from the shallower sediments; or there is a greater rate of CH<sub>3</sub>Hg degradation in the shallow

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#### Upper mesospheric wind structure in Antarctica

UNDER a joint Indo-Soviet agreement I was the first Indian scientist to winter in Antarctica during the 17th Soviet Antarctic Expedition, 1971-73. In particular, I participated in the meteorological rocket soundings of the upper atmosphere carried out at Molodezhnaya, a station located at 67° 40'S, 45° 51'E at a height of 42 m above mean sealevel in Enderby Land, East Antarctica (Fig. 1).

In 1972 the mean annual temperature observed at the station was  $-10.5^{\circ}$  C varying from a lowest minimum of -35.8° C to a highest maximum of 7.0° C. South-east winds prevailed at the surface with frequent gusts of 21-41 m s<sup>-1</sup>. Annual mean relative humidity was 65% and the annual mean precipitation observed was 0.14 cm.

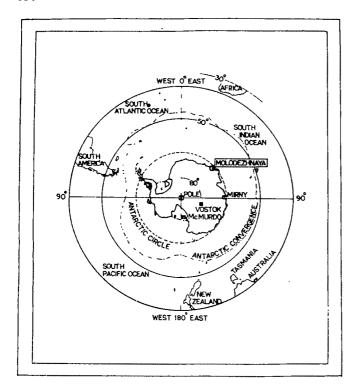


Fig. 1 Map of Antarctica and surrounding area showing the location of the station Molodezhnaya.

While I was in Antarctica, 60 M-100 meteorological rockets were launched from Molodezhnaya and 16 of these carried an additional wind sensor ('chaff') for determining the mesospheric winds. Most of the flights were successful and the average rocket apogee reached was 86.93 km. A summary of the chaff-borne flights, evenly distributed throughout 1972,

is given in Table 1. The results derived for the region 50–90 km (mostly for the 60–80 km layer in the upper mesosphere) are presented here. This is the first meteorological study of the upper mesospheric winds carried out in Antarctica.

The chaff used consisted of cylindrical aluminium-coated glass fibres having a diameter of about 0.025 mm. About 400 g of chaff, in a special container divided into two parts, was ejected at rocket apogee. The descending chaff cloud was then tracked by a high sensitivity Meteor-2 radar. Table 1 gives the corresponding wind tracks of all such flights. At the time of ejection at rocket apogee, above 80 km, the chaff fell at a high speed, about 160 m s<sup>-1</sup>, but as it descended further it slowed down to about 4 m s<sup>-1</sup> at around 56 km because of the greater density and viscosity of the air at lower altitudes.

The radar data on the drift of the trajectory of the chaff are used to measure the wind speed and direction in the mesospheric region under study. Corrections for changes in the wind with altitude are also applied. The accuracy in measuring the wind speed at higher altitudes is about 6-10 m s<sup>-1</sup>. Time-height cross sections for zonal and meridional winds are plotted using the conventional interpolation method in Figs 2 and 3.

The zonal components over Molodezhnaya, Antarctica, show that in the upper mesosphere the winds were predominantly easterly in the summer half and westerly in the winter half. In January and February (southern summer, Fig. 2) the speeds of the easterly winds ranged from 10 to 50 m s<sup>-1</sup> in the 60-80 km region for which the chaff data were available. On January 26, 1972, however, the zonal wind structure was found to have a small core of weak westerlies with wind speed about 10-15 m s<sup>-1</sup> in a narrow altitude region from 77 to 85 km. In February the easterly winds were stronger and attained a maximum speed of 96 m s<sup>-1</sup> at 82 km. During March there was only one rocket flight and during April there were none. During the first week of May, westerly winds of about 20 to 60 m s<sup>-1</sup> were detected. During the second week of May, however, the winds again became strong easterlies with a maximum speed of 113 m s<sup>-1</sup> at 66 km and a secondary maximum of 79 m s<sup>-1</sup> at 80 km.

Table 1 Summary of the 16 launchings of rockets containing a chaff sensor from Molodezhnaya, Antarctica, in 1972								
Date	Time (дмт)	Rocket apogee (km)	Wind track (km)		60–70 km MsCI <sub>65</sub> (m s <sup>-1</sup> )	65–75 km MsCI <sub>70</sub> (m s <sup>-1</sup> )	70-80 km MsCI <sub>75</sub> (m s <sup>-1</sup> )	75–85 km MsCl <sub>80</sub> (m s <sup>-1</sup> )
January 5	1450	82.80	80-62	Meridional Zonal	N/A N/A	6.1 -40.1	2.4 -41.5	N/A N/A
January 19	1440	84.65	84–64	Meridional Zonal	N/A N/A N/A	3.3 -38.2	5.5 -29.3	-2.5 -16.4
January 26	1435	88.03	8662	Meridional Zonal	N/A N/A	-14.9 -33.3	-16.4 -13.1	-6.7 $-6.2$
February 16	1535	83.70	82–60	Meridional Zonal	-3.4 $-21.2$	-2.6 -26.7	5.1 -30.5	N/A N/A
March 1	0330	87.50	86–56	Meridional Zonal	9.3 -12.4	9.3 -26.3	14.5 33.9	12.8 -46.0
May 3	1400	88.00	84–56	Meridional Zonal	1.9 29.0	-12.7	-24.2 2.3	6.4 -21.1
May 6	1410	91.50	90-58	Meridional Zonal	3.4 45.0	20.9 27.7	-3.9 33.0	-6.2 19.6
May 17	1750	87.94	8456	Meridional Zonal	-31.1 -61.5	-17.8 $-57.0$	-50.1 -37.5	-44.0 $-9.5$
June 21	1400	92.85	88–56	Meridional Zonal	-5.8 54.2	-8.1 34.2	-18.2 13.6	-25.6 14.0
June 28	· 1405	88.40	84-54	Meridional Zonal	29.7 89.2	23.0 60.6	5.4 16.8	-6.4 17.2
July 5	1400	86.70	84–54	Meridional Zonal	12.1 116.6	17.9 74.0	14.7 32.5	16.2 16.3
July 19	1400	85.30	84–56	Meridional Zonal	10.1 32.4	0.4 21.7	-12.7 38.9	-0.9 41.3
September 6	1400	88.44	82–62	Meridional Zonal	-15.6 36.2	-28.0 0.9	0.2 10.4	N/A N/A
September 20	1405	86.55	66-52	Meridional Zonal	4.3 50.9	N/A N/A	N/A N/A	N/A N/A N/A
October 4	• 1353	86.50	80-58	Meridional Zonal	6.7 12.8	4.3 4.7	3.1 -1.6	N/A N/A
December 20	1430	81.98	80–64	Meridional Zonal	N/A N/A	10.6 -36.3	7.5 -38.7	N/A N/A

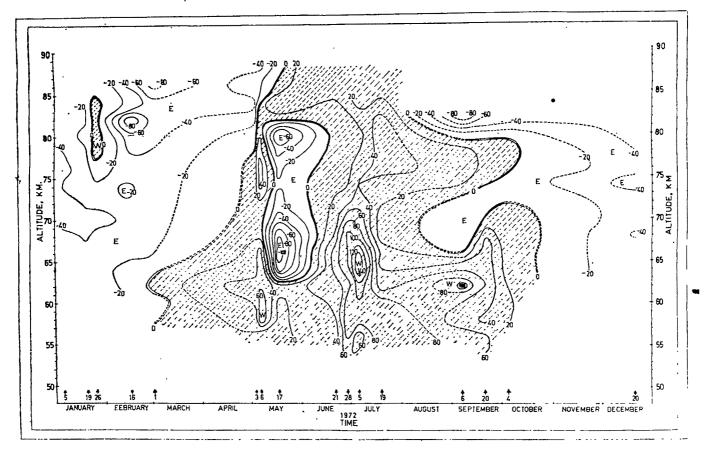


Fig. 2 Time-height cross section for Molodezhnaya zonal winds (1972) with west (+, shaded areas) and east (-) components in m s<sup>-1</sup>.

---, Extrapolated data. Arrows above the abscissa show the date on which data were obtained.

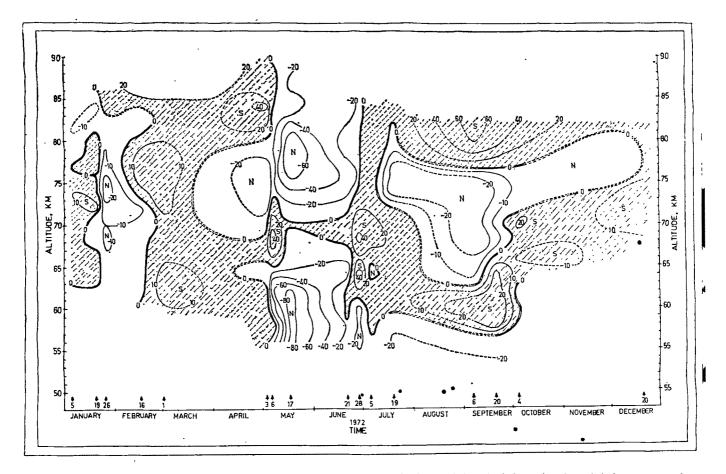


Fig. 3 Time-height cross section for Molodezhnaya meridional winds (1972) with south (+, shaded areas) and north (-) components in m s<sup>-1</sup>. ---, Extrapolated data. Arrows above the abscissa show the date on which data were obtained.

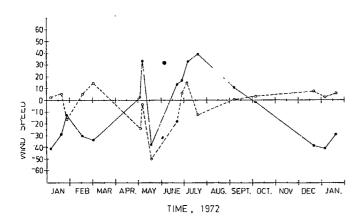


Fig. 4 Zonal flow (—) obtained by averaging winds from the individual soundings over a 10-km layer centred at 75 km (Mesospheric Circulation Index, MsCI). ---, Meridional flow. Positive values refer to west and south components.

Wind speeds in m s<sup>-1</sup>.

In June and July (southern winter) a westerly jet was formed which attained a maximum speed of 157 m s<sup>-1</sup> at 64 km and became weaker at higher altitudes. The strong westerly winds started to weaken in September-October (southern spring) in the 60-70 km region and weak easterlies started developing above 70 km. There were no ascents with chaff payloads during August and November. In December the zonal flow was characterised by easterlies of about 20-40 m s<sup>-1</sup>.

The meridional cross section of the winds shows that they were of variable nature (Fig. 3). In January weak southerly winds of about 5–15 m s<sup>-1</sup> predominated in the upper mesosphere. In February the winds were mainly northerlies, ranging from 5–25 m s<sup>-1</sup>. In May the northerly winds became stronger and formed a jet which had a maximum speed of 88 m s<sup>-1</sup> at around 60 km, with a secondary maximum of 79 m s<sup>-1</sup> at 78 km. In the narrow region 65–70 km there was a small core of southerlies having a maximum speed of 57 m s<sup>-1</sup> at 68 km.

In June and July the northerly winds changed to southerly ones with a maximum speed of 48 m s<sup>-1</sup> at 64 km. In September and October the winds were mainly southerlies of about 10–60 m s<sup>-1</sup> with lower values at around 60 km and higher values at around 82 km. There was a core of weak northerlies at around 75 km. In December the meridional flow was characterised by weak southerlies of about 5–15 m s<sup>-1</sup>.

The seasonal reversal of winds at different times of the year can be shown clearly by a method devised by Webb¹. In analysing the Meteorological Rocket Network (MRN) data Webb¹ calculated an average flow over a layer 10 km thick centred at 50 km and called it the Stratospheric Circulation Index (SCI) of the appropriate layer. Adopting the same method, I have computed mean values of the wind speeds in m s⁻¹ obtained from the individual soundings by averaging over a 10-km layer centred at each of the altitudes 65, 70, 75 and 80 km (Table 1). Since this presents an average flow of the mesosphere under study, it is termed the Mesospheric Circulation Index (MsCI). Its values for the 70–80 km layer centred at 75 km, MsCI, are plotted in Fig. 4.

In conclusion, I found that zonal winds were predominantly easterly in the summer half and westerly in the winter half whereas the meridional winds were variable. From the analysis of the MsCI (Fig. 4) it is obvious that the summer easterly flow changed to a westerly flow in the first week of May, followed by a rapid reversal to an easterly flow in the second week. The easterly flow again changed to westerly flow in mid June. The meridional flow (Fig. 4) also showed a rapid shift

from northerlies to southerlies in the last week of June. The southerly flow again rapidly changed to northerly flow in mid July. These rapid shifts in both zonal and meridional components indicated a sudden 'explosive' change in temperature distribution which might have occurred in the upper mesosphere. I am at present investigating this phenomenon. The winter westerly flow changed to the summer easterly flow at about the end of September. It seems that both for the zonal and the meridional flows the summer—winter shift was a rapid and dramatic change, whereas the winter—summer shift was slow and unspectacular. I am also studying diffusion phenomena in the mesosophere using the chaff data.

I acknowledge the collaboration of the members of the 17th Soviet Antarctic Expedition, 1971–73, and thank the Hydrometeorological Service of the USSR and the Department of Atomic Energy, Government of India, for allowing me to take part in the expedition, and giving me the privilege of being the first Indian Explorer of Antarctica, which was made possible by the late Professor Vikram A. Sarabhai. I also thank Professor P. R. Pisharoty for stimulating guidance and encouragement.

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# Flow near an oscillating cylinder in dilute viscoelastic fluid

There are many natural phenomena in which nonlinear interactions of time-dependent inputs give rise to steady—that is, time-independent outputs. One of these is a steady streaming belonging to a class of secondary flows sometimes called acoustic streaming. It occurs when a circular cylinder oscillates normal to its axis in an unbounded Newtonian fluid<sup>1-3</sup>. We report here on the steady secondary flow induced when a long thin cylinder oscillates as described in a viscoelastic liquid. We found that the direction of steady streaming is opposite to that found for the bulk of fluid when the experiment is performed with a Newtonian fluid.

Reversal of secondary flow has been noted in several flows<sup>4-6</sup> when fluid has been altered from Newtonian to viscoelastic behaviour.

Our experimental apparatus (Fig. 1) was adapted from that used by Bertelson et  $al^3$ . The oscillating brass cylinder was 6.72 cm long, and had a diameter of 1.6 mm. It was suspended on two bronze leaf springs, each 0.15 mm thick. The oscillating system was contained in a cylindrical glass container 9.55 cm long and 7.05 cm in diameter. The glass container was mounted in a strong, fixed magnetic field. Oscillation was induced by an alternating current (Fig. 1).

The container was filled with test fluid containing tracer particles (hollow glass spheres  $40-60~\mu m$  in diameter). Pathlines representing steady secondary streaming were obtained by photographing particles in a plane perpendicular to the axis of the vibrating cylinder. Only the secondary flow appears in the photographs because illumination is provided by a stroboscopic source synchronised with the frequency of oscillation.

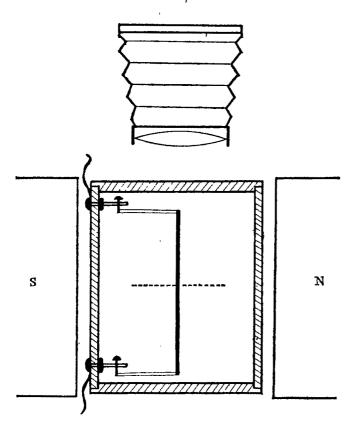


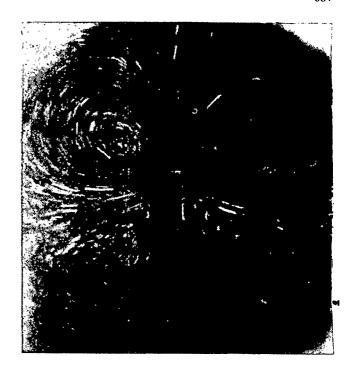
Fig. 1 Schematic diagram of apparatus. Dotted line indicates plane of illumination. S and N, Magnets.

Photographs were first taken with a Newtonian test fluid: a 50% by weight solution of glycerine in water. The viscosity of the liquid was approximately  $6 \times 10^{-3}$  N s m<sup>-2</sup>. The oscillating frequency was set at 45 Hz. For small amplitudes of oscillation (approximately 25% of the cylinder diameter) the results shown in Fig 2a were obtained. There are two regions of flow (see ref. 3). In the inner region near the oscillating cylinder the pathlines form small closed vortices with fluid flowing toward the oscillating cylinder in the direction of oscillation and away from it in the transverse direction (Fig. 2b). Of more predominance is the outer region which also contains closed vortices but with an opposite sense of rotation. Thus, the predominant secondary flow is away from the oscillating cylinder along the axis parallel to the direction of oscillation. (At lower Reynolds numbers a thicker inner vortex system can be obtained?.)

The contrast is striking when the results of Fig. 2 are compared with those of a dilute viscoelastic fluid possessing drag reducing properties<sup>8</sup>. The secondary flow shown in Fig. 3 was obtained under conditions similar to those cited for Fig. 2 with one exception. The glycerine-water solution contained 0.01% by weight of Dow Separan AP30, a water-soluble polyacrylamide. No small inner vortex is visible in Fig. 3, and the predominant secondary flow is toward the oscillating cylinder along the axis parallel to the direction of oscillation. These results could signify that the small inner vortex observed with the Newtonian fluid is greatly enlarged in the experiments performed with polymer solution.

In Figs 2a and 3a there is an asymmetry between upper and lower portions of the photographs. That is because the cylinder motion was not truly rectilinear, but followed an arc determined by the radius of the spring supports. The experiment of Fig. 3a was performed at a slightly higher amplitude causing more predominant curvature.

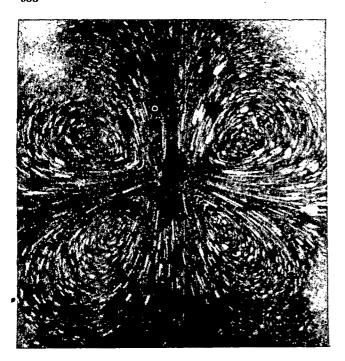
As the addition of 100 p.p.m. of Separan has little effect on the shear viscosity of the glycerine-water solution, the drastic change in secondary flow probably results from the elasticity of the Separan solution.



 $\boldsymbol{a}$ 

Fig. 2 Steady secondary flow for Newtonian fluid: 50% by weight glycerine in water. Frequency of oscillation, 45 Hz. a, Tracer photographs at f/2.8 and 1 s exposure time, showing inner and outer vortices; b, sketch drawn from a showing direction of circulation. Double arrow, direction of cylinder oscillation.

b



Further experiments with varying amounts of Separan showed that the reversal of flow persisted to very dilute solutions. Secondary flow characteristic of Newtonian flow only reappeared when the Separan was reduced to 10-20 p.p.m.

Hopefully, this new phenomenon may be helpful for characterisation and, perhaps, further elucidation of the nature of dilute solutions of drag reducing polymers.

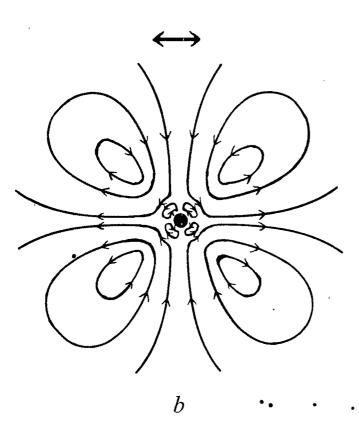
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a

Fig. 3 Steady secondary flow for viscoelastic fluid: 50% by weight glycerine in water, with 0.01% by weight of Dow Separan AP30. a, Tracer photographs at f/2.8 and 1 s exposure time, showing absence of identifiable inner vortex analogous to Fig. 2; b, sketch drawn from a showing direction of circulation.

## Taylor vortex instability and annulus length effects

THE stability of the flow between rotating cylinders has both fundamental and technological significance. The classical analysis by G. I. Taylor<sup>1</sup> of the stability of the circumferential laminar flow deals with coaxial cylinders for which the length L is infinite and the radial clearance  $c=R_2-R_1$ , is small, but subsequent work2 has eliminated the need for the small clearance approximation. The flow, once unstable, takes the form of axisymmetric toroidal vortices and these Taylor vortices can in turn become unstable with the onset of non-axisymmetric wavy vortices. Early analysis of this instability³ was again restricted to coaxial cylinders of infinite length and small clearance, but Eagles4 has produced a stability analysis avoiding the small clearance approximation and has applied it to a cylinder radius ratio  $R_1/R_2=0.951$ . No stability analyses seem to exist for an annulus of finite length and most experimental workers choose to work with long cylinders even though short cylinders are more likely to be encountered in engineering applications. Recently I described some observations<sup>5</sup> of vortex sizes in short annular clearances and I noticed the apparent absence of wavy vortices in those experiments: now I have more positive evidence of the considerable effect of annulus length on the wavy vortex critical speed.

The present flow visualisation results are for a rotor of diameter 91.42 mm concentrically positioned inside a Perspex stator of bore 103.2 mm, thickness 19 mm and effective length 250 mm, so that  $R_1/R_2=0.894$ . Vortices in the test fluid, a silicone oil of kinematic viscosity v=19.3 centistoke, were made visible by adding a small quantity of aluminium particles of typical maximum dimensions 0.01 mm. Given a high standard of control of rotor speed (N revolutions s-1) and fluid temperature, this commonly-used visualisation technique enabled the critical speeds to be determined repeatedly to better than

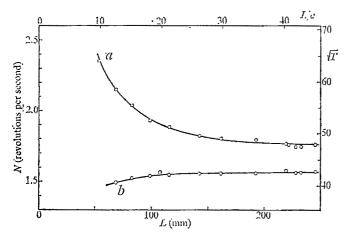


Fig. 1 Effect of annulus length L on critical speeds N determined from visual observations. a, Wavy vortices; b, Taylor vortices.  $R_1/R_2 = 0.894$ .

 $\pm 0.5\%$  in general, with no hysteresis apparent for speed increasing and decreasing. Annulus length was varied by changing the depth of the test fluid, and the end boundary conditions were asymmetric, with the lower end a stationary solid surface and the upper a free liquid surface. Table 1 shows that observed critical speeds are in satisfactory agreement with calculated values for the maximum annulus length tested. Figure 1 shows that length has a small effect on the speed at which Taylor vortices appear but a marked effect on the speed at which wavy vortices appear: at L/c=25 the second critical speed is about 10% higher than the calculated value and at L/c=10 about 40% higher.

To achieve greater length: clearance ratios, observations were also made using a rotor of diameter 94.45 mm inside a stator of diameter 100.01 mm, giving a radius ratio  $R_1/R_2$ 0.954. The silicone oil viscosity was v=6.10 centistoke at the controlled operating temperature. Here, critical speeds were determined from measurements of the torque reaction on the stator which was supported on externally-pressurised air bearings: the onset of Taylor vortices produced an upward discontinuity in the slope of the torque-speed relationship as speed was increased in small steps and the onset of wavy vortices produced a later and less pronounced downward discontinuity. This method also gave good results, repeatable to better than  $\pm 0.5\%$  at larger lengths but with slightly diminished accuracy at short lengths where the slope changes became somewhat rounded. For L/c < 10, the Taylor critical in particular was less certain as the torque-speed relationship became nonlinear and slightly concave upwards before the major change of slope occurred: the latter was taken as the critical

**Table 1** Dimensionless critical speeds  $\sqrt{T} = \frac{(2\pi N R_1 c)}{y} \frac{1}{2c/(R_1 + R_2)}$ 

$\sqrt{1 - \left[ \left( \frac{2\pi i \sqrt{K_1 c}}{\sqrt{V}} \right) \right] \sqrt{\left[ \frac{2c}{K_1 + K_2} \right]}$						
Fig. no.	1	2				
Experimental method	Visualisation	Torque measurement				
Cylinder radius ratio $(R_1/R_2)$ Taylor vortex onset speed:	0.894	0.954				
Observed $\sqrt{T_c}$	42.7 at $L/c = 45.1$	41.9 at $L/c = 106.6$				
Calculated $\sqrt{T_c}$	42.8 at $L/c = \infty^*$	41.8 at $L/c = \infty^*$				
Wavy vortex onset speed:						
Observed $\sqrt{T_1}$		55.1 at $L/c = 17.3$				
		45.2 at $L/c = 106.6$				
Calculated $\sqrt{T_1}$	45.8 at $L/c = \infty \ddagger$	44.0 at $L/c = \infty \uparrow$				

By interpolation from Walowit, Tsao and Di Prima<sup>2</sup>.

condition. The results, shown in Fig. 2, are generally similar to those in Fig. 1 although the very small variation in the Taylor critical speed with length is of opposite sign to that in the visualisation experiment. The critical speed for the onset of wavy vortices begins to rise appreciably as L/c values are reduced below 40 and the maximum value measured was about 22% above the minimum. Table 1 shows that observed critical speeds at the maximum L/c value of 107 are in satisfactory agreement with theoretical values.

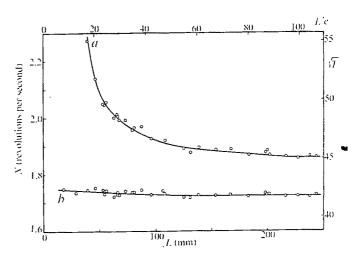


Fig. 2 Effect of annulus length L on critical speeds N determined from torque measurements. a, Wavy vortices; b, Taylor vortices.  $R_1/R_2 = 0.954$ .

These visualisation and torque observations at two different cylinder radius ratios agree in indicating little effect of annulus length on the critical speed for the onset of Taylor vortices but considerable effect on the critical speed for the onset of wavy vortices. The length; clearance ratio needed to avoid a rise of more than say 5% in the second critical speed is about 35 but more measurements are required before a precise estimate can be made. Additional preliminary experiments suggested that a similar limit applies when the cylinders are eccentrically positioned, eccentricity raising both critical speeds. Evidently the assumption of infinite length in stability theory is far less satisfactory for the wave instability than for the Taylor instability, and the observation by Coles<sup>6</sup> on the relationship between the two critical speeds requires re-examination in terms of length effects.

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<sup>†</sup>By interpolation from Eagles<sup>4</sup> (with m=1).

<sup>‡</sup>By extrapolation (so value suspect) from Eagles4.

#### Early turbulence and drag reduction phenomena in larger pipes

SOLUTIONS of certain high molecular weight polymers have been shown to exhibit the phenomena of both early turbulence1-7 and drag reduction4.5.8 depending on whether the measurements were made under laminar flow conditions for the former case or under turbulent flow conditions for the latter. In general, early turbulence has been observed only in capillary tube flows while drag reduction has been observed in a variety of flow geometries, the only requirement being a developing or developed turbulent flow field.

A correspondence of sorts between early turbulence and drag reduction was surmised earlier<sup>9</sup> and was also strongly implied by capillary tube measurements where a continuous transition between early turbulence onset points and drag reduction onset had been observed. A close relationship between the two phenomena was therefore suggested<sup>5</sup>. Specifically, the non-Newtonian onset point (either early turbulence or drag reduction) for 1 p.p.m. solutions of Polyox coagulant in a series of salt solutions were found to be described by the following empirical relationship in these capillary flows

$$\tau^* = (78.7)/(1 - 1.39M)$$

where  $\tau^*$  is the non-Newtonian onset point and M the molarity of the magnesium sulphate salt solution.

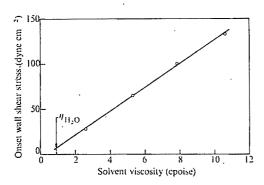


Fig. 1 Onset of early turbulence for 100 p.p.m. WSR-205 solutions in glycerine-water mixtures. Pipe diameter, 0.553 cm.

The suggestion has been made<sup>2</sup> that early turbulence might also be observed for flows in larger diameter tubes provided sufficiently high wall shear stresses could be attained in the laminar flow regimes. This hypothesis was confirmed by subsequent experiments in larger diameter pipes with drag reducing additives dissolved in water-glycerine mixtures<sup>6,7</sup>; the glycerine serving the function of significantly altering the solution viscosity, thus providing the needed higher wall shear stresses in laminar flow conditions. Figure 1 shows the variation of the onset wall shear stress for early turbulence with solvent viscosity for 100 p.p.m. WSR-205 solutions in glycerine-water mixtures in a 0.553 cm pipe. The plot is essentially linear over the range of solvent viscosities chosen. The linear relationship suggests that an extrapolation might justifiably be made to the pure water case. Thus, a hypothetical value of 7.4 dyne cm<sup>-2</sup> is obtained for the onset of early turbulence in this particular pipe for the water case had the flow not undergone transition to the turbulent regime. Data<sup>8</sup> are also available for the same WSR-205 sample under turbulent flow conditions in pure water. Figure 2 shows a plot of the drag reduction onset wall shear stress against the log concentration. Although 100 p.p.m. WSR-205 had not been studied in the earlier work a short extrapolation

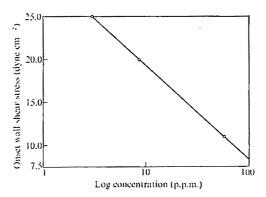


Fig. 2 Onset of drag reduction against log concentration for WSR-205 solutions in turbulent flow. Pipe diameter, 0.660 cm.

of the linear plot in Fig. 2 to the 100 p.p.m. value yields an onset wall shear stress for drag reduction of approximately 8 dyne cm<sup>-2</sup>. Although both sets of data rely on short extrapolations this additional evidence does seem to support earlier claims4,8,9 that drag reduction and early turbulence are somewhat related phenomena.

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### Preserved amino acids from silicified protein in fossil Radiolaria

THE discovery of amino acids in fossils1 has stimulated a wide range of biochemical studies<sup>2,3</sup> over the past 20 yr with implications for evolution, geochronology, and biomineralisation. One productive line of evolutionary investigation has been the analysis of calcified protein from Recent shells of calcareous invertebrates such as molluscs<sup>4,5</sup> and planktonic Foraminifera<sup>6,7</sup>. Results indicate that the amino acid composition is speciesspecific and varies in a systematic manner which parallels morphology. Moreover, data from extinct 18-Myr-old Foraminifera suggest that the technique can be applied directly to the deepsea fossil record for establishing phylogenetic affinities6.7. Virtually nothing is known, however, of the nature of the organic matrices which may exist in fossil skeletons composed of silicia rather than calcium carbonate. Amino acid data from this study are therefore believed to be the first evidence reported for the presence and diagenesis of silicified protein in the fossil record.

Radiolaria, important contributors to the siliceous ooze characteristic of Equatorial Pacific and Antarctic regions, were

Table 1 Amino acid composition of Recent skeletal protein\*

Amino acid	Spun	Radiolaria ( nellaria - <i>S. glacialis</i>	Nassellaria	a Mean	Recent Foramini- fera (calcified) <sup>7</sup> Mean of 16 species
Asp	151	154	167	156	212
Thr	50	40	43	44	82
Ser	80	40	70	63 .	84
Glu	115	135	134	127	114
Gly	193	299	192	227	138
Ala	88	81	100	90	100
Val	82	48	82	71	60
Met	6	3	< 1	5	9
Allo-Ileu	4	3 3 25	3	5 3	
Ileu	29	25	28	27	36
Leu	50	58	68	59	44
Tyr	13	11	20	15	23
Phe	31	30	30	30	35
His	1	3 5	< !	2 9	20
Orn	10	.5	11		-
Lys	53	49	14	39	22
Arg	44	16	38	33	21
Total	•				
Concentrat (nmol/g ske	(1,355)	(1,602)	(1,360)	(1,439)	(2,523)

<sup>\*</sup> Residues per 1,000 amino acid residues.

Core samples were disaggregated in a dilute sodium hexametaphosphate solution with ultrasonic vibration, washed with distilled water on a 62 µm sieve, and air dried. Although the skeletons of these planktonic protozoans are generally small (50-500  $\mu m$ ), certain species were found to be sufficiently large and abundant to allow manual extraction from the coarse fraction under a binocular microscope. Samples from aggregate species were exhaustively cleaned ultrasonically in a series of sodium hexametaphosphate washes and distilled water rinses, dissolved in cold hydrofluoric acid, evaporated to dryness, and hydrolysed in 6 N HCl in sealed tubes under nitrogen at 110° C for 22 h. Hydrolysates were evaporated to dryness in a vacuum centrifuge and stored in a freezer until analysed. Suitable species were not found in sufficient abundance for replicate analyses and results are therefore based on single analyses. Analyses of sub-mg monospecific collections of low-density skeletons (2-5 µg per skeleton) were made possible with a Durrum D-500 amino acid analyser operated in manual mode at the pmol ( $\sim 10^{-10}$  g) level of sensitivity. Reproducibility is within  $\pm$  5% based on replicate analyses of standard amino acid mixtures. Results in Tables 1 and 2 are corrected for reagent blank values and hydrolytic losses of Thr and Ser.

the first group of silica-secreting organisms chosen for study. All species analysed are members of the Polycystina group which are currently defined as "Radiolaria with skeletons of opaline silica without admixed organic compounds", based on earlier microscopic evidence, Before this study, only the Tripylea group was thought to contain skeletal organic matter. Tripyleans are rarely preserved in deep-sea sediments because of their fragile structure.

Two of the three Recent species analysed (Spongoplegma antarcticum Haeckel and Spongotrochus glacialis Popofsky) were isolated from the top of an Antarctic deep-sea core (RC12-229), the third (Cyclampterium neatum Sanfilippo and Riedel) from the top of an Equatorial Pacific core (RC12-66). Down-core species (Cyclampterium neatum and Dictyastrum angulatum Ehrenberg) were separated from RC12-66 at the palaeomagnetic boundary of Gilbert/Epoch 5 which corresponds to an age of 5.1 Myr.

A protein-containing organic matrix was found in all three species of Recent Radiolaria (Table 1). With the exception of cystine, all amino acids normally found in protein hydrolysates were present. Proline was detected, but not quantified, with the 590-nm photometer in the analyser. Two additional amino acids, p-alloisoleucine and ornithine, were found in significant amounts indicating that some diagenesis had occurred in the few thousand years represented by the core top samples.

Each species has a unique composition with inter-species variations being greater than experimental error for 16 of the 17 amino acids. Glycine, aspartic acid, glutamic acid, and alanine are the four most abundant amino acids in all three species and account for 60% of all residues.

The only notable difference between the two spumellarian species and the single nassellarian is a 73% lower abundance of lysine in the nassellarian skeletons. The Spumellaria and the Nassellaria are the two major taxonomic groups within the Order Polycystina<sup>8</sup>.

Total amino acid concentration in silicified radiolarian skeletons is about half the content of calcified foraminiferal shells (Table 1). Significant differences are also observed in the proportion of certain amino acids although the four most abundant acids in both mineral types are glycine, aspartic acid, glutamic acid, and alanine. The relative abundance of aspartic and glycine is reversed in the two groups with glycine being greater in Radiolaria (aspartic second), whereas aspartic acid ranks first in

Table 2 Diagenetic changes in amino acid content of radiolarian skeletons over 5.1 Myr

Amino acid	Recent S. glacialis (nmol g <sup>-1</sup> )	Spumellaria 5.1 Myr D. angulatum (nmol g <sup>-1</sup> )	Ratio 5.1 Myr/Recent	Recent C. neatum (nmol g <sup>-1</sup> )	Nassellaria 5.1 Myr C. neatum (nmol g <sup>-1</sup> )	Ratio 5.1 Myi¶Recent
Asp	246.4	27.4	0.11	226.9	10.8	0.05
Thr	64.8	<del>-</del>	<del></del>	58.4	5.0	0.09
Ser	64.0	9.7	0.15	95.0	13.9	0.15
Glu	216.8	81.4	0.38	181.9	24.3	0.13
Gly	477.6	145.7	0.31	260.9	31.6	0,12
Ala	129.3	93.0	0.72	135.3	25.8	0.19
Val	76.5	57.6	0.75	111.9	12,5	0.11
Met	5.3	<del></del>		Trace		_
Allo-Ile	5.3	11.2	2.11 .	4.4	1.9	0.43
Ile	39.6	16.4	0.41	38.4	2.7	0.07
Leu	93.5	27.9	0.30	92.2	11.8	0.13
Tyr	17.1	<del></del>	-	26.6	Trace	_
Phe	48.3	15.3	0.32	40.9	8.8	0.22
His	5.1	<del></del>		Trace	_	
Orn	7.8	365.5	46.86	15.3	• 11.1	0.73
Lys	78.3	8.6	0.11	19.4	3.0	0.15
Arg	26.3	-		52.2	_	
Totals	1,602.0	859.7	0.54	1,359.7	163.2	0.12

Foraminifera (glycine second). Other major compositional differences are 77% more lysine, 46% less threonine, 35% less tyrosine, and 34% more leucine in radiolarian skeletons.

Approximately 75% of the total amino acid content is peptidebound. Analyses of non-hydrolysed samples of Recent Spongoplegma antarcticum and Spongotrochus glacialis show total 'free' amino acid concentrations amounting to 25% of the hydrolysed values. As Recent planktonic Foraminifera show less than 5% 'free' amino acid content (K.K., unpublished), diagenesis of proteins seems to be accelerated in siliceous skeletons. Experiments with Recent foraminiferal protein indicate that the hydrofluoric acid dissolution step does not contribute significantly to the observed hydrolysis in Radiolaria.

Comparative analyses of samples from Recent and 5.1-Myrold species (Table 2) indicate that the protein-containing organic matrix undergoes severe diagenesis over geological time. An 88% reduction in amino acid content was found when the 5.1-Myr-old sample of nassellarian Cyclampterium neatum was compared with a Recent sample. A substantial reduction (46%) was also observed in the pair of spumellarians. If the diagenetic product, ornithine, is excluded from the comparison, the effect is more similar for the two groups, with reductions of 69% (Spumellaria) and 89% (Nassellaria). Scanning electron micrographs suggest that the substantially higher diagenetic rate in the nassellarian is related to a greater susceptibility to solution because of a nodulose skeletal surface11.

The 'free' amino acid content of a non-hydrolysed 5.1 Myr nassellarian sample represents only 19% of the total content on hydrolysis, whereas 77% of the acids from a hydrolysed spumellarian sample of the same age is accounted for by 'free' acids11. These results suggest that the bulk (80%) of the remaining few amino acids in the nassellarian are in bound form and may represent either well-protected intact protein or humic acid-type polymers from in situ reactions12.

The diagenetic reductions of 69% (Spumellaria) and 89% (Nassellaria) in 5.1-Myr-old samples are in marked contrast to an average reduction of 40% observed in 18-Myr-old Foraminifera6,7. Factors which may contribute to the accelerated diagenetic rate of Radiolaria are (1) the high internal water content (up to 20% by weight) of opaline silica<sup>13</sup> promoting the hydrolysis of peptide bonds, and (2) greater skeletal porosity leading to more rapid attack and leaching by external pore waters in the sediment.

Contamination of fossil materials by amino acids in the sediment is always a potential problem because of the ubiquitous nature of these compounds. As a test for surface contamination in situ, samples of S. antarcticum and S. glacialis were treated with concentrated (30%) hydrogen peroxide for 1 h before normal sample preparation. No significant differences in composition were observed between these samples and those cleaned with only sodium hexametaphosphate.

The presence of a protein-containing matrix in radiolarian skeletons provides a rationale for extending amino acid studies to siliceous fossils. Promising lines of investigation would include the dating of siliceous sediments by the epimerisationracemisation reactions of preserved amino acids14,15 and the tracing of evolutionary lineages by the analysis of speciesspecific amino acid patterns7.

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### Search for selectivity between optical isomers in reactions of polarised positive muons with alanines and octanols

ONE of the most intriguing problems in chemical evolution is the origin of optical asymmetry in biopolymers. The easiest way to state the problem is: why are proteins made almost exclusively of L-amino acid optical isomers and natural sugars of D-optical isomers? That proteins must be made of only one kind of optical isomer is understandable on the basis of their need for precise three-dimensional conformations in order to perform their catalytic roles as enzymes. But, is it just a matter of chance, as suggested in refs 2-4, that our proteins are L-, or is there (was there) some asymmetrical agent on our planet that made the protein L-configuration the one upon which life is based?

One possible 'non-chance' explanation stems from the unequal decomposition of optical isomers5.6 and the appearance of optical activity in the products of light-mediated reactions<sup>7-10</sup> in circularly polarised light. A slight excess of right-circularly polarised light in sunlight reflected and/or refracted in the Earth's magnetic field<sup>11</sup> could produce overall optical asymmetries. But laboratory experiments have detected such effects only for high intensities of circularly polarised light; this mechanism has therefore been seriously questioned as an explanation for the origin of optical asymmetry in biological molecules1.

Table 1 Residual muon polarisation in various media							
Target	Residual asymmetry (A)	Residual polarisation* (Pres)					
Distilled water	$0.153 \pm 0.003$	$0.55 \pm 0.03$ (def.)*					
0.67 M racemic alanine in water	$0.151 \pm 0.004$	$0.54\pm0.03$					
Solid L-alanine	$0.089 \pm 0.003$	$0.32\pm0.02$					
Solid D-alanine	0.089 ± 0.003	$0.32 \pm 0.02$					
Liquid L-2-octanol	$0.140 \pm 0.003$	$0.50 \pm 0.03$					
Liquid D-2-octanol	$0.140 \pm 0.002$	$0.50\pm0.03$					

<sup>\*</sup> Residual polarisation derived from  $P_{\text{res}} = A/A_{\text{o}}$ , where  $A_{\text{o}} = 0.278 \pm 0.016$  is calculated by comparing  $A(\text{H}_2\text{O})$  with the independently determined residual polarisation in water,  $P_{\text{res}}(\text{H}_2\text{O}) = 0.55 \pm 0.03$  (ref. 18).

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A second possible 'non-chance' explanation invokes spinpolarised electrons and associated bremsstrahlung emitted in the  $\beta$ -decay of certain natural radioactive nuclides<sup>12,13</sup>. One report claims that in aqueous solution D-tyrosine is decomposed by <sup>90</sup>Sr  $\beta$  emission faster than L-tyrosine<sup>14</sup>. This report has been questioned by later papers<sup>15,16</sup>, however, and must be considered unsubstantiated.

This paper is concerned with a search for optical selectivity in the interaction of a new agent, the polarised positive muon, with organic molecules. The degree of spin polarisation in muon beams produced from decaying pions is typically about 80% (refs 12 and 13). As far as we are aware, this is a higher degree of polarisation than that of any particles previously used in the search for selectivity of interactions with optical isomers. Since polarised muons are known components of cosmic rays<sup>19</sup>, a selective interaction, possibly leading to selective destruction of one optical isomer, might provide another 'non-chance' explanation for the particular appearance of L-amino acids and D-sugars in living cells.

Positive muons, upon slowing down, form the neutral atom 'muonium' ( $\mu^+$  e<sup>-</sup>, or Mu)<sup>20</sup> which is analogous to the hydrogen atom, except for its mass,  $m_{\rm Mu}=0.1131m_{\rm H}$ . Because of the high degree of polarisation of the  $\mu^+$  'nucleus', the formation of the muonium atom and/or its subsequent chemical reactions might vary between optical isomers. We have, however, been unable to detect any optical selectivity in the interactions of muonium with either (1) solid D- and L-alanine or (2) liquid D- and L-2-octanol.

The  $\mu^+$  beam of the Berkeley 184-inch cyclotron was used in these experiments. The quantity actually measured was the 'residual polarisation' of muons stopped in the various substances. Although the muons enter the target with a well-defined spin polarisation, their polarisation after stopping is dramatically dependent upon the chemical properties of the medium in which they come to rest.

The spin polarisation of the muons is easily detected through their decay products: the muon decays by way of

$$\mu^+ - \cdot e^+ \, \nu_e \, \overline{\nu}_u$$

with a mean lifetime of 2.2  $\mu$ s. The positron (e<sup>+</sup>) from the decay is, on the average, about twice as likely to be emitted in the direction of the  $\mu^+$  spin as in the opposite direction<sup>17,18,21</sup>. An ensemble of polarised positive muons thus broadcasts its polarisation in a shower of fast (up to 50 MeV) positrons. By detecting these positrons in scintillation counters, we monitor the magnitude of the  $\mu^+$  polarisation. In the present experiments, a perpendicular magnetic field is applied, causing the  $\mu^+$  spin to precess at its Larmor frequency. A counter telescope fixed in the plane of precession is most likely to detect a positron if the muon decays when its spin points towards the telescope; thus the e<sup>+</sup> detection probability in that telescope will rise and fall as the muon polarisation sweeps past it<sup>18</sup>.

The measured amplitude of the resultant time oscillation of the positron detection probability is called the experimental asymmetry, A, and is proportional to the residual polarisation of the  $\mu^+$ ,  $P_{res}$ 

$$A = A_0 P_{\rm res}$$

The constant of proportionality  $A_0$  is an empirical constant unrelated to the chemical properties of the medium<sup>22,23</sup>. The values of  $P_{res}$  obtained in the present work are given in Table 1. Since water has been thoroughly studied<sup>22,23</sup>, the value of  $P_{res}$  (H<sub>2</sub>O) = 0.55  $\pm$  0.03 from refs 22, 23 is used to calibrate the results listed in Table 1. A more detailed technical description of the apparatus and experimental technique can be found in refs 18, 22 and 23.

Each target consisted of about 500 g of the substance under study. The solid alanines (A grade) were obtained from Calbiochem, La Jolla, California, and the liquid octanols from Norse Laboratories, Santa Barbara, California. All samples were used without further purification.

In most condensed media, any residual polarisation observed through muon precession is the result of chemical reactions of muonium with molecules of the medium; in the absence of such reactions, all the muon polarisation is lost within a few nanoseconds through the so-called "muonium mechanism". This depolarisation mechanism arises after the formation of muonium and is due to the contact hyperfine interaction between  $\mu^+$  and  $e^-$  magnetic moments, which couples the two spins together so as to reverse the  $\mu^+$  spin within  $\sim 10^{-10}$  s. Fast precession of muonium in the external magnetic field conspires with this hyperfine coupling to effectively depolarise the  $\mu^+$  in muonium within  $\sim 10^{-9}$  s. The only way a muon can be spared this fate is if the Mu atom that it forms reacts chemically with a molecule of the medium to incorporate the  $\mu^+$  into a diamagnetic molecule.

A rigorous theoretical description of the mechanism of  $\mu^+$  depolarisation is found in refs 22–24. Briefly, there are two sorts of chemical reactions of Mu that prevent complete depolarisation of the  $\mu^+$ : (1) 'normal' thermal reactions, which occur after the Mu atom has thermalised and started depolarising the  $\mu^+$ ; and (2) epithermal or 'hot atom' reactions, which occur while the Mu atom is still slowing down, long before any depolarisation has taken place. The latter reactions are assumed to be important in the energy region from  $\sim 10$  eV down to thermal energies, and are usually the dominant channel for reactions of Mu in all but the most reactive substances. In water or methanol, for instance, the residual polarisation is believed to be due exclusively to hot atom reactions<sup>22,23</sup>.

As can be seen from Table 1, the residual polarisation in a 0.67 M solution of racemic alanines in water is the same, within experimental uncertainties, as that observed in pure water. We conclude that there is little, if any, thermal reaction of Mu with alanine molecules, at least in solution 22,23. Taken with the observation that Mu has a large epithermal reaction probability in most organic substances, this suggests that the residual polarisation in the solid alanines is primarily a result of hot atom reactions of muonium. The same assumption can be made for the octanols, on the somewhat weaker grounds that Mu has a large, purely epithermal reaction probability with methanol 22,23.

The data in Table 1 show that there is no significant difference between the reaction probabilities of polarised Mu atoms with enantiomers of alanine and 2-octanol. The errors given are derived from the fitting procedure (see refs 22 and 23). Although it is statistically possible that a few per cent difference could be realised ( $\Delta A/A = 0.0 \pm 0.05$  for alanine and  $\Delta A/A = 0.0 \pm 0.03$  for 2-octanol), this difference could only be determined accurately on the basis of more measurements. Remembering that it is the  $\mu^+$  nucleus of the Mu atom that is polarised (negligible polarisation is transmitted to the electron in the time of  $\sim 10^{-12} \, \mathrm{s}$  that muonium takes to thermalise), it is perhaps not surprising, in retrospect, that the hot atom reactions are optically indiscriminate.

A more sensitive test of optical selectivity of polarised muons may be possible if muonium precession can be observed directly in these substances. Apparently, recent results using positrons<sup>25</sup> suggest that electrons picked off by muons to form Mu atoms might be expected to have an optically-influenced polarisation. This effect would be reflected in first order in the amplitude of muonium precession signals. The feasibility of such studies is now being considered.

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#### Bilayers at the air—water interface?

FILMS of octadecanol and the corresponding C<sub>18</sub> acid (stearic acid) appear to occupy 10° A2 per molecule at the air-water interface when spread from ethanol or acetone, as opposed to 20 A°2 per molecule when spread from a (non-polar) solvent such as hexane or benzene. Ries1 has proposed that in a polar or non-polar spreading solvent the amphipathic molecules adopt a bimolecular configuration with the hydrophilic groups pointing, respectively, outward and inward. It was further postulated that when spread at an air-water interface, only the latter could be 'unzippered' by the substrate to form a monolayer. Spreading from a polar solvent would thus result in bilayer formation and explain the apparent loss of half the film molecules. In view of the upsurge of interest in membrane and model membrane systems, the possibility of forming a lipid bilayer at the air-water interface was certainly exciting and clearly warranted further investigation.

Films of L-α-dipalmitoyl phosphatidylcholine (DPPC), 1-monoolein and stearic acid (Applied Science Laboratories; purities > 99 %) were selected for study. DPPC and 1-monoolein are examples of substances which do, and do not, readily form a bilayer2. Isotherms and surface potentials for spread films of these three substances are shown in Figs 1-3. Shifting from hexane to ethanol resulted in a substantial decrease in the apparent area per molecule for all three films, the extent of the decrease being dependent on the nature of the film molecules when other factors were held constant. The large shift to smaller areas per molecule occurs between 90 and 100 volume per cent ethanol (Fig. 4a). Between 0 and 90 mol per cent ethanol, the

already liquid-expanded isotherms become slightly more expanded at low surface pressures and the surface potentials appear to decrease slightly (Fig. 1), both effects indicating the presence of small amounts of ethanol in the substrate<sup>4</sup>. Contrary to Ries1, the large shift in areas per molecule was sensitive to changes in solute concentration (Fig. 4b) and even to the rate of delivery at the air-water interface. In effect, a 50% decrease in apparent area per molecule can be obtained simply by selecting the correct spreading solute concentration for that particular component. Of particular interest, is the surface potential which showed little change on transition from monolayer to 'bilayer'. In addition both phase changes and compressibility are independent of the apparent physical state of the film. These latter observations led us to question seriously the assigned bilayer configuration for films spread with polar solvent and to attempt to respread an original stearic acid 'bilayer' as a monolayer using a non-polar solvent.

The respread isotherms initially revealed gross contamination and great care was required before hexane-respread films (originally spread either from hexane or ethanol) were impurity free. When

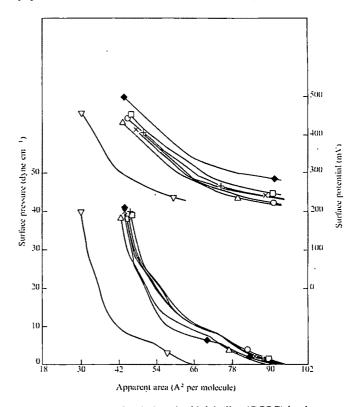


Fig. 1 L-α-Dipalmitoyl phosphatidylcholine (DPPC) isotherms at the air-water interface (pH 6 substrate) at 22° C. Films spread at the air—water interface (pri o substrate) at 22°C. Finits spread from various mixtures of ethanol and hexane. Percentage ethanol:  $\phi$ , 10;  $\Box$ , 44;  $\bigcirc$ , 50;  $\times$ , 60; +, 70;  $\triangle$ , 90;  $\bigcirc$ , 100. Isotherms and surface potentials were continuously recorded. The upper curves are the surface potentials. Spreading solvent concentration approximately 0.15 mg DPPC per ml solvent. Ethanol and hexane were column purified and distilled before use. Hydrochloric acid used in the aqueous substrate was also distilled. Water was double-distilled from glass (the first time from alkaline permanganate) and subsequently double-distilled from quartz. An automated continuous recording teflon trough film-balance was used, which together with initial film spreading techniques, has been described elsewhere<sup>3</sup>. Techniques were developed for the removal, concentration, solution and respreading of initially spread films after it was found that adding Ing of initially spread films after it was found that adding solvent to a spread monolayer or bilayer clearly indicated solvent retention. Scrupulous care in cleaning glassware, trough and solvents were required for reproducibility in such experiments. Reproducibility for initially spread films was  $\pm$  0.5 A<sup>2</sup> per molecule for a given surface pressure ( $\pi$ ) and  $\pm$  10 mV for surface potential values. Reproducibility in areas per molecule on respreading from ethanol a film initially spread from hexane was approximately  $\pm$  5% at a value of 15% less than the originally spread film. The initial spread film was never compressed beyond 15 dyne cm<sup>-1</sup>. Respreading films with hexane, originally spread from ethanol, gave values with some 10% or less film loss.

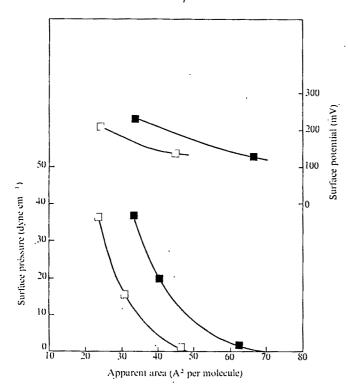


Fig. 2 1-Monoolein isotherms at the air—water interface spread from ethanol (☐) and hexane (☐) at 21.5° C. (pH 6 substrate). Spreading solvent concentration approximately 0.1 mg 1-monoolein per ml solvent.

this was achieved (Fig. 3), respread areas per molecule were usually slightly lower ( $\sim 10\,\%$ ) than the original film values. For films previously spread from ethanol, slightly high areas per molecule were obtained by removing significant amounts of the aqueous substrate with the original film. Only when high solute concentrations were used, were areas per molecule obtained for a respread film that were slightly greater than those for the original 'bilayer'. Immediately after film removal the substrate appeared clean, but after an hour or so, however, trace amounts of stearic acid were once again obtained. These additional amounts did not arise from collapsed material

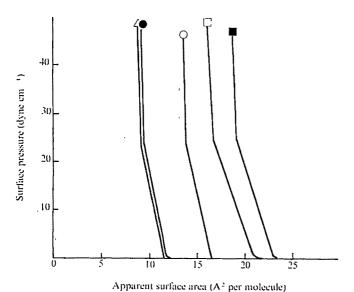
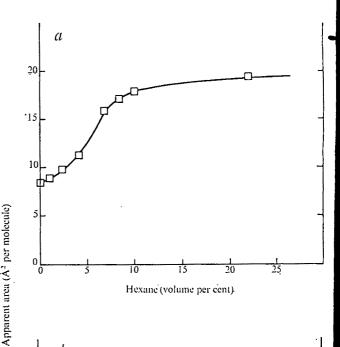


Fig. 3 Stearic acid films at the air-water interface spread from hexane ( $\blacksquare$ ) and respread with hexane ( $\square$ ); from ethanol ( $\bullet$ ) and respread with hexane ( $\triangle$ ); from ethanol at  $pH\ 2\ (\bigcirc$ ). Substrate at  $pH\ 6$  except where indicated. Initial spreading solvent concentration approximately 0.21 mg stearic acid per ml solvent.

at the trough edge. Finally, addition of acid to the substrate before initial spreading from ethanol led to areas per molecule intermediate between those of a 'bilayer' and a monolayer (Fig. 3). A similar, though smaller, effect was obtained by adding salt to the substrate.

From the above, we conclude that spreading of a film with a polar solvent does not result in the formation of a bilayer, but in fact leads to the loss of a significant amount of material, the precise amount depending on the nature of the film material, spreading solvent, the substrate pH and ionic strength, and also on the spreading solvent concentration and rate of deposition. The effect of the substrate additives indicates that film loss occurs through the substrate.

We have observed two phenomena which help explain these results: (1) Substantial film loss is accompanied by gross Teflon trough contamination. A clean trough permits rapid, complete water drainage. After multiple ethanol depositions and film



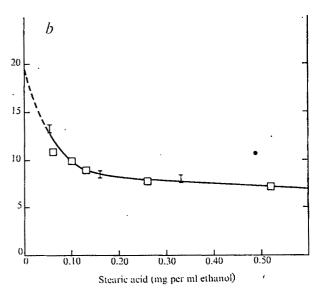


Fig. 4 a, Apparent area per molecule of stearic acid as a function of spreading solvent composition. A 0.29 mg stearic acid per ml ethanol solution was diluted with hexane to give the stated ethanol-hexane ratio prior to spreading at 20.5° C. b, Apparent area per molecule of stearic acid as a function of its concentration in ethanol at 20.5° C. The zero abscissa value was obtained by spreading from hexane. Points marked (1) were obtained by at least three determinations. Points marked (1) were obtained by preparing the most concentrated solution and then carrying out a series of dilutions.

loss, water film retention on the trough was greater than normal. (2) Schlieren patterns are readily observed during ethanol spreading at the air-water interface, penetrating deeply into the water. This behaviour is greatly reduced by the addition of small amounts of hexane. We postulate, therefore, that most of the lost film material is trapped at the Teflon surface. The well known porosity of Teflon provides a substantial surface area for this purpose. Certainly it is not located at the air-water

We conclude that spreading from a polar solvent results in substantial film loss but that the residual film is still a monolayer; the near identical surface potential, phase changes and compressibility, independent of spreading solvent, all support this conclusion.

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## *In vivo* nuclear fission in the aetiology of decompression sickness

THERE can be little doubt that gas bubbles accompany and may well cause decompression sickness, as the effect of recompression is usually to alleviate the symptoms' and to eliminate the new reflectors of ultrasound which appear in man and animals during decompression<sup>2-5</sup>

As gas bubbles do not form in supersaturated fluids or animals without gas micronuclei<sup>6,7</sup> we assume that the body of a normal man must contain microbubbles, which may expand at decompression to give the observed effects. Furthermore, it seems that such microbubbles must be created continually. Some studies of bubble formation in vivo<sup>8-16</sup> assume implicitly the presence of pre-existing micronuclei, which evades the problem.

One mechanism for the production of micronuclei in man, tribonucleation, has been suggested by Ikels<sup>11</sup>. We suggest here that some gas micronuclei are created in man by spontaneous nuclear fission (SF) in vivo. Ionising particles dissipate energy along their tracks and if the linear energy transfer (LET) to a metastable liquid is sufficiently high, the resultant 'thermal spike'12 may nucleate bubbles which can subsequently grow to visible size, as in the bubble chamber.

Water is rarely used in bubble chambers13, though persistent microbubbles should be created in cold water which is sufficiently supersaturated with gas and subjected to radiation.

As we were unable to find any report of radiationinduced nucleation of aqueous solutions supersaturated with gas leading to bubbles of visible size, we have studied the stability of solutions of two  $\alpha$ -active radioisotopes in cold, supersaturated water.

Polypropylene outer containers from Brunswick disposable 1 ml syringes (capacity 12 ml) were filled with stabilised distilled water saturated with air at 215 pound inch<sup>-2</sup> gauge (14.7 bar), (see ref. 15). The top 2 ml of liquid was removed from each tube, 1 ml of solution (Table 1) was introduced, and the tube was topped up with liquid paraffin and sealed by a thin polythene sheet. The tubes then received high hydrostatic pressure treatment to eliminate any gas micronuclei introduced with the additions. The final gas tension in the amended tubes was about 13.1 bar. The tubes were then removed for observation at atmospheric pressure for 6 h. If visible bubbles appeared in any tube during this period it was recorded as unstable; the other specimens were recorded as stable.

Most of the control tubes remained stable for the full 6 h and the tubes with the lead salt were no less stable than the controls (Table 1). We may, therefore, conclude that a heavy metal ion does not necessarily promote bubble formation. The tubes containing uranium were, however, much less stable than the controls, the difference being statistically significant ( $\chi^2 = 208$ , P < 0.0005).

To distinguish between the effects of the principal mode of radioactive decay for 238 U (α emission) and the much less frequent spontaneous nuclear fission, thorium tubes were prepared. The quantity of salt was arranged so that the  $\alpha$ emission was the same as in the uranium tubes, and the  $\alpha$ energies are similar. The SF halflife of 232Th (ref. 16) is so much greater than that for 238U (ref. 17) that the probability of one event for all 100 thorium tubes in 6 h is less than  $10^{-2}$ .

The thorium tubes were only marginally less stable than the controls (Table 1); the difference between the thorium and uranium results is again highly significant ( $\chi^2=93$ , P < 0.0005). We conclude, therefore, that the nuclear fragments which are liberated by SF of 238U will nucleate bubbles which can grow to visible size in cold supersaturated water.

It may not be widely appreciated that spontaneous nuclear fission occurs in man. The phenomenon seems to occur, however, sufficiently often to maintain a stock of stable gas micronuclei in an average man, and exceptionally it may promote bubble formation during one decompression.

The principal isotope must be 238U, of which the body of an average man in the UK contains about  $110 \,\mu g$  (ref. 18).

		results	٠.	Weight of	f addition	α	activity	SF	SF
	Stable proportion	Sta	able (%)	Salt	Isotope	pCi	dis s -1	Disintegration h <sup>-1</sup>	Disintegration (600 h)
Control (water) Pb(NO <sub>3</sub> ) <sub>2</sub> <sup>238</sup> U* <sup>282</sup> Th†	262/287 • 95/100 56/200 87/100	•	91.3 95.0 28.0 87.0	none 1,000 mg 8 mg 41 mg	4.6 mg 13.6 mg	1.5 1.5	56 56	$\begin{array}{c} 9.2 \times 10^{-2} \\ < 2.8 \times 10^{-6} \end{array}$	55 <1.6 × 10 <sup>-3</sup>

<sup>\*</sup> Uranium oxalate. † Thorium nitrate.

If the SF halflife of 238U is 1018 yr (ref. 17) man should experience a disintegration about once every three weeks.

A new nucleus might not survive compression, But if each disintegration during decompression, or during the preceding period at raised ambient pressure were to cause an attack of decompression sickness, a large group of men working eight-hour shifts at pressure should experience a bends rate of about 2.2% of man-decompressions. At the Tyne Road Tunnel men were working for some months at pressures of 27-29 pound inch-2 gauge. Of the 5,465 mandecompressions recorded after shifts of 8 h or more at these pressures, some 118 resulted in an attack of bends<sup>19</sup>, giving a rate of 2.16%.

One mechanism for the stabilisation of new nuclei is an 'organic skin'20,21, though in our case high temperatures are involved so fission-induced cavities may well be surrounded by a coagulavelum<sup>22</sup> of denatured protein. Certainly, an abundant supply of suitable macromolecules is to be found in most body fluids. The body burden of uranium resides mostly in bone<sup>18,23</sup>. The commonest symptom of acute decompression sickness (the bends) is described as a diffuse pain associated with bones, and the most important chronic consequence of hyperbaric exposure is Caisson disease of bone. In dogs, the ends of the long bones retain more uranium than the shaft<sup>23</sup>, and both in dog<sup>23</sup> and in man<sup>24</sup> the element is mainly found on the surfaces of cancellous bone and the endosteum. Both are sites associated with the lesions of Caisson disease of the femur and humerus.

There can be considerable geographical variation in the total  $\alpha$  activities of human bone ash samples<sup>23</sup>. If a similar variability in the uranium content—and therefore in the formation rate of fission-induced nuclei-could be discovered and correlated with the observed variation in individual susceptibility to decompression sickness, it may become possible to prove or refute our hypothesis. From our comprehensive records of diving experience and work in compressed air26 it is easy to select men who are particularly resistant or susceptible, though estimation of the body burden of uranium in live men without occupational exposure is less easy. The traditional method is to determine the uranium content of urine<sup>27,28</sup>, and despite uncertainty in relating the excretion rate to body burden, it seems probable that body burdens may be compared usefully in this wav.

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#### Pleistocene date for man in Tasmania

THE Pleistocene colonisation of Tasmania has long been predicated1,2 but no dated human occupation sites of that age have been reported before. The excavation of a cave site on Hunter Island<sup>3</sup>, 6 km off the coast of north-western Tasmania (40° 34'S, 144° 45'E; Fig. 1) has yielded the first radiocarbon date of Pleistocene age from a human occupation site in Tasmania.

The Cave Bay Cave is a large sea cave in a slate cliff containing an undisturbed deposit rich in faunal remains and with signs of human occupation. In a preliminary cutting 1.75 m deep, the top 25 cm were found to contain abundant evidence of man: hearths, lenses of marine shell and quartz flakes were present, and the bones of seabirds predominated. Below this level, evidence of human occupation was sparse. The deposit was still rich in faunal remains but seabirds were absent whereas native rodents and small marsupials were

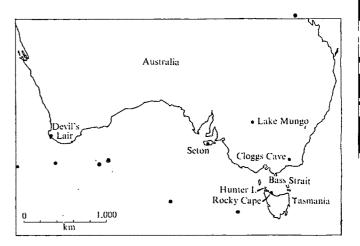


Fig. 1 Southern Australia.

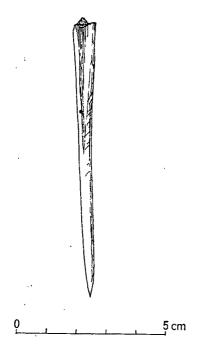


Fig. 2 Bone point found in Pleistocene deposits in Cave Bay

abundant. There were also some larger marsupials, some of which are not now found on Hunter Island, including the native cat (Dasyurus cf. viverrinus). A bone point and two pieces of flaked quartz-convincing evidence of the presence of man-were found between 80 and 100 cm below the surface. The bone point is a ground and polished piece of macropod fibula (Fig. 2). Associated charcoal has been carbon dated to  $18,550\pm600$  b.p. (ANU-1361).

The final maximum of the last glaciation was responsible for lowered sea levels about 18,000 yr BP and Hunter Island would have been a hill on a land bridge between Tasmania and the Australian mainland4.

Similar sites in southern Australia with Pleistocene dates, bone artefacts and good faunal preservation are Clogg's Cave at Buchan in south-eastern Australia (17,720 ± 840 b.p., ANU-1044)5, the Seton site on Kangaroo Island, South Australia (16,100 $\pm$ 1,000 b.p., ANU-1221; ref. 6 and R. J. Lampert, personal communication), and the Devil's Lair, Western Australia (24,600 ±800 b.p., SUA-31)7. All of these sites have been the haunt of non-human predators who have contributed their own share of faunal remains. In the Cave Bay Cave, the abundant remains of rodents and small marsupials in the lower levels were probably deposited as pellets regurgitated by owls, and the native cat has probably contributed the bones of larger animals such as bandicoots and possums, which show distinct signs of non-human gnawing.

At Clogg's Cave, the Seton site and the Devil's Lair, there is evidence of the presence of the Tasmanian wolf and devil (Thylacinus cynocephalus and Sarcophilus harrisii) although there is so far no sign of them at the Cave Bay Cave. The immediate difficulty generated by the presence of these carnivores is that of discriminating the food remains of man from those of the other predators.

A more intriguing problem is suggested by a comparison between these sites and post-Pleistocene cave sites; for instance, the Rocky Cape sites of north-western Tasmania<sup>1</sup>. There, a total of 6 m of shell midden deposit has built up over the last 8,000 yr and there is no evidence of the presence or action of predators other than man (ref. 1 and Rhys Jones, personal communication). There is generally much sparser evidence of human occupation at the older sites in terms of artefacts and stratigraphic features than at Rocky Cape. It seems that Pleistocene man in southern Australia made less intensive use of cave and shelter sites than his post-Pleistocene descendants. Another important human occupation site of Pleistocene age and with faunal remains preserved is at Lake Mungo-an open site where people camped on the beaches of a lake8.

The Cave Bay Cave has implications not only for an understanding of the ancestry and development of the unique Tasmanian culture2, but also for the ecology of Pleistocene man in Australia.

This investigation is part of a project supported by the Australian National University<sup>3</sup>.

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## Identification of metabolic dechlorination of highly chlorinated biphenyl in rabbit

CHLOROBIPHENYLS with one to four chlorine atoms per molecule are metabolised by certain animals, plants and microorganisms but the more highly chlorinated biphenyls are rather resistant to metabolic change<sup>1</sup>.

Organochlorine compounds may be identified at low concentrations in crude extracts of natural samples by a high resolution mass spectrometric method involving photoplate detection2-4. The method is based on the facts that first, in conditions of high resolution each ion of unique molecular composition is recorded individually and, second, the relatively large mass deficiencies (the actual mass being lower than the closest nominal mass) of both chlorine isotopes. The accurate mass for <sup>35</sup>Cl for instance is 34.9688 which accounts for a mass deficiency of  $-31.1 \times 10^{-3}$  mass units (m.u.), the the corresponding value for 37Cl being -34.1×10<sup>-3</sup> m.u. These mass deficiencies cause the exact mass of ions which contain these atoms to be at lower masses than ions of the same nominal mass which contain only atoms (that is C, H, N, O) present in most biological molecules. Thus, the high resolution photoplate technique is capable of detecting chlorine-containing mass deficient ions in crude biological extracts. As the photoplate acts as an integrating ion detector it is possible to identify compounds present in low concentrations (\leq 0.01 p.p.m.).

This method has now been applied to the unambiguous identification of metabolites from organochlorine compounds' including the detection of trace components. After the usual dosing of the animal, urine, faeces or organs to be examined were extracted with a suitable solvent and a small portion (10-20  $\mu$ g) of the dried extract was placed in the mass spectrometer sample tubes. Mass spectra from the sample were then obtained by exposing photoplates to ions derived from the crude extract for up to 30 min or

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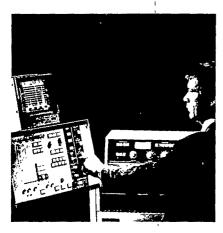
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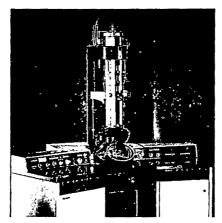
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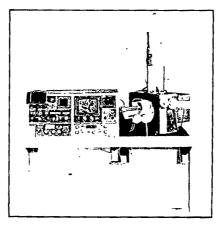
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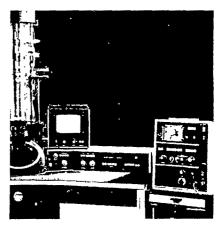
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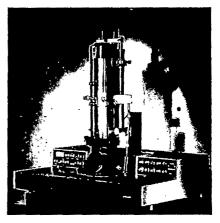


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Proceedings of the Third International Congress of Plant Tissue and Cell Culture held at the University of Leicester, Leicester, England, 21st-26th July, 1974

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In this volume, reviews by nineteen international experts cover the full range of this recent work, each review introducing a particular field of study in which plant tissue and cell culture techniques have proved of key importance. They indicate the most active areas of current research and also the barriers to immediate progress, whether these lie with the need for better experimental systems or for advances in the techniques of culture.

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This book provides a systematic development of geometrical optics and the optical aberration theory needed for optical design. The author provides only that material which is useful to designers with access to computers. Methods designed for outmoded computational techniques and those simply not well adapted for practical design purposes have been ignored, and the author has emphasised finite methods. This is one of the first books on aberration theory which is aimed mainly at working optical designers, and while intended primarily as a reference work, it contains some material which has previously only been published in research journals.

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longer, each at different temperatures (fractional distillation). The developed photoplates were then screened for metabolites (mass deficient ions) manually or by using an automatic photoplate reader coupled with a computer.

Rabbits were fed a diet of food pellets containing 1% 2,2',4,4',5,5'-hexachlorobiphenyl. The feeding time was 7 d and the total concentration of chlorobiphenyl administered was 1 g kg<sup>-1</sup>. The urine, which was collected during the entire feeding period, was extracted with ether and the extract treated in the manner described earlier. Manually measured data (perfluorokerosene as internal standard, experimental values < 10 p.p.m. from calculated values) showed the presence of two major metabolites (Fig. 1, I, II) formed from the hexachlorobiphenyl by hydroxylation and hydroxylation with concomitant dechlorination respectively. A third, minor, metabolite corresponding to a molecular composition IH was detectable only in a limited number of photoplate exposures at about 60° C.

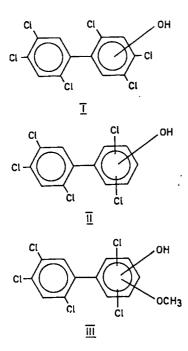


Fig. 1 Metabolites of 2,2',4,4',5,5'-hexachlorobiphenyl.

Figure 2 shows the partial mass spectrum (enlarged photoplate record) of the crude ether extract exposed for 10 min at 60° C. The very mass deficient ions (> 0.1 m.u.) corresponding to the organochlorine metabolites are seen, clearly resolved at lower mass in each cluster of lines corresponding to ions of the same nominal mass. At m/e 370 and m/e 372 the molecular ion (M<sup>+</sup>; that is molecular weight calculated with <sup>35</sup>Cl) and M+2 ion for compound III are visible. Measurement of the exact mass of the 37Cl isotope peak (M+2 ion) further confirmed the composition of the compound. At m/e 374 in addition to the weak M+4 peak of compound III a strong line corresponding to compound I is evident. At m/e 375 and m/e 376 lines for the <sup>13</sup>C and <sup>37</sup>Cl isotope peaks of I are obvious.

The advantages of this method in the study of metabolism of organochlorine compounds seem to be first, that no radioactive labelling is necessary because the chlorine atoms act as tracer, second, the method is simple and rapid with unambiguous results so that large scale screening is possible, and third, the danger of loss of metabolite or degradation during separation procedures is minimised and non-volatile products can be detected much more readily than by GC-MS. The usefulness of the method may be con-

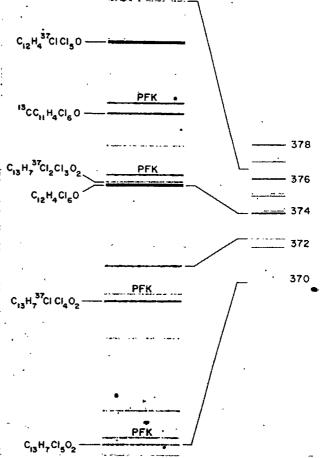


Fig. 2 Partial mass spectrum (m/e 370-376; enlargement)of photoplate record) of crude ether extract from rabbit urine. Animals were dosed with 2,2',4,4',5,5'-hexachloro-biphenyl. Lines corresponding to compound III are C<sub>13</sub>H<sub>7</sub>Cl<sub>3</sub>O<sub>2</sub>(M<sup>+</sup>; m/e 370); C<sub>13</sub>H<sub>7</sub><sup>37</sup>Cl Cl<sub>4</sub>O<sub>2</sub>(M<sup>+</sup>+2; m/e 372). Lines corresponding to compound I are C<sub>12</sub>H<sub>4</sub>Cl<sub>6</sub>O(M<sup>+</sup>; m/e 374); C<sub>12</sub>H<sub>4</sub> <sup>37</sup>Cl Cl<sub>3</sub>O(M<sup>+</sup>+2; 376); <sup>13</sup>CC<sub>11</sub>H<sub>4</sub>Cl<sub>6</sub>O(<sup>13</sup>C isotope peak of M<sup>+</sup>; m/e 375). Lines marked with PFK correspond to ions derived from the mass-marker perfluxors. correspond to ions derived from the mass-marker perfluorokerosene. All unmarked lines correspond to mass positive ions from coextracted natural products.

siderably improved in this respect by the use of field desorption techniques.

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### Variation in assortative mating in two colonies of Arctic skuas

THE Arctic skua is a polymorphic seabird with pale, intermediate and dark plumaged phenotypes. Sexual selection of the phenotypes takes place when pairs are being formed; during the breeding season the darker males generally mate before the paler males<sup>1,2</sup>. This gives them a selective advantage because a pair which breeds earlier in the season will fledge a greater average number of chicks<sup>1,3</sup>. Computer models have been used to estimate the females mating preferences for the darker males<sup>3</sup>.

Assortative mating also takes place between the phenotypes. In previously published data<sup>4</sup>, which were taken from observations on several colonies by different ornithologists, the intermediates and darks were not distinguished from each other and were lumped into a single class of dark birds. Some colonies showed assortative mating with a significant excess of pale  $\times$  pale and dark  $\times$  dark matings compared with the frequencies to be expected if pairing were random. Other colonies showed no deviation from random mating. These differences in assortative mating are not statistically significant, however: an orthogonal analysis gives  $\chi^2 = 10.09$  with 6 degrees of freedom corresponding to P = 0.13. In this paper we give new evidence of assortative mating, showing that in one colony pale birds mate assortatively while in another intermediates mate assortatively.

Table 1 Observed numbers and theoretical frequencies of matings of Arctic skuas on Fair Isle and Foula in Shetland

Mating type	of ma	ed no. tings Foula	. , Theoretical frequencies
Pale $\times$ pale	7	23	$\alpha u + [(u^2(1-\alpha)^2)/(1-\alpha u - \beta v - \gamma w)]$
Pale × inter	24	30	$2uv(1-\alpha)(1-\beta)/(1-\alpha u-\beta v-\gamma w)$
Pale × dark	12	22	$2uw(1-\alpha)(1-\gamma)/(1-\alpha u-\beta v-\gamma w)$
Inter × inter	48	42	$\beta v + \left[ v^2 (1-\beta)^2 / (1-\alpha u - \beta v - \gamma w) \right]$
Inter × dark	17	38	$2vw(1-\beta)(1-\gamma)/(1-\alpha u-\beta v-\gamma w)$
Dark × dark	8	10	$\gamma w + \left[ w^2 (1-\gamma)^2 / (1-\alpha u - \beta v - \gamma w) \right]$
Total no.	116	165	1 .

To derive the theoretical frequencies, it is assumed in the model that the pale, intermediate and dark phenotypes occur in the proportions u, v and w and mate assortatively with another bird of the same phenotype in the proportions  $\alpha$ ,  $\beta$  and  $\gamma$ . Thus the proportion of all birds that mate at random is  $1 - \alpha u - \beta v - \gamma w$ .

Research on the colony of Arctic skuas on Fair Isle began in 1948 and continued until 1962. In 1973 we started further researches in order to analyse the components of natural and sexual selection which the earlier research had revealed. In 1974 we also surveyed the Arctic skuas on Foula, an island in the Shetlands 66 km north-west of Fair Isle. The survey was

carried out by systematically quartering the breeding grounds and observing which pair of birds attacked or feigned injury in a particular area. The data of the numbers of breeding pairs are shown in Table I with theoretical frequencies given by a model of assortative mating analysed previously. In the model, a proportion of the birds of each phenotype mate assortatively with others of similar phenotype; the remaining birds mate at random. Pale, intermediate and dark birds mate assortatively in the proportions  $\alpha$ ,  $\beta$  and  $\gamma$ . Female preferences generally determine assortative matings, but the model allows for both male and female preferences.

If the intermediates and darks in the data of Table I are lumped together, the analysis of  $\chi^2$  is similar to the analysis of the data published earlier. There is a significant overall deviation from random mating, but not in the Fair Isle data alone. This difference between the colonies is not itself significant. If the intermediates and darks are separated, however, the data of both Fair Isle and Foula show very significant deviations from random mating, but in different directions. The parameters  $\alpha$ ,  $\beta$  and  $\gamma$  of the general model of assortative mating have been fitted to the data by maximum likelihood. The effects on χ<sup>2</sup> of fitting the different parameters are shown in Table 2. Fitting the single parameter  $\beta$  to the Fair Isle data reduces the value of  $\chi^2$  to less than the degrees of freedom.  $\chi^2$  is further reduced, but not of course significantly, by fitting the additional parameter y. Fitting the parameter a to the Foula data similarly reduces  $\chi^2$  to an insignificant value and fitting  $\beta$  reduces it still further. Our data are thus explained if 47% of intermediates mate assortatively on Fair Isle and 32% of pales mate assortatively on Foula.

These inferences are confirmed by the log likelihoods and support limits given in Table 3. Unless  $\beta$  is fitted to the Fair Isle data and  $\alpha$  to the Foula data, the log likelihoods are always more than two units of support below the maximum. The support limits show the precision of the maximum likelihood estimates of these parameters.

What might explain the differences we have found and what consequences may follow? O'Donald4 suggested that imprinting might be the explanation but found there was no evidence for imprinting in data on the matings of parents and the subsequent matings of their chicks. If imprinting were a cause of the females' mating preferences, it would produce neither the strong assortative mating of intermediates on Fair Isle, nor the difference between the colonies. An alternative explanation would be a genetic difference between the colonies in mating behaviour. This might have arisen by chance as a 'founder effect', or evolved by selection if pairs differ in breeding success on the islands. Birds fledged in one colony sometimes breed in other colonies, however; and if this migration is random, it must reduce any genetic differences between colonies. The variation in assortative mating thus presents a remarkable evolutionary problem for future researches.

Assortative mating has the effect of reducing heterozygosity.

Table 2	Estimation of	the parameters	$\alpha$ , $\beta$ and $\gamma$ in t	he mo	dels of asso	rtative mating fo	or the Arctic ski	as of Fair Isle a	nd Foula
Parameters estimated	Arctic skuas of Fair Isle ML estimates			Arctic skuas of Foula ML estimates					
	_	ameters	$\gamma^2$		d.f.	of par	ameters	χ <sup>2</sup>	d.f.
None		_	9,950		3	· -		11.556	3
α	0.1	102	9.110		2	0.3	316	1.923	2
β	0.4	174	1.385		2	0.2	276	6.802	2
γ	0,2	237	5.797		2	0.0	)15	11.501	2
α and β	0.0	0.475	1.385	•	Ī	0.288	0.180	0.538	1
$\alpha$ and $\gamma$	0.089	0.233	5.485		ī	0.315	0.0	1.923	1
$\beta$ and $\gamma$	0.437	0.165	0.111		1	0.275	0.0	6.801	1

The maximum likelihood (ML) estimates were found by calculating the log likelihood (base e) for a given set of values of the parameters. Random variations in the values were then introduced until a higher log likelihood was obtained. This process was repeated with smaller and smaller random variations until the maximum likelihood was reached. Explicit solutions of the equations for maximum likelihood can be obtained if in addition to the phenotypic frequencies only one parameter is to be estimated. The explicit solutions give the same values as those found by the random search.

Table 3 The log likelihoods and support limits of the estimated parameters

a,	Arctic skuas of Fair Isle Parameters estimated	Maximum la	a likalihaad
	i arameters estimated	Maximum lo	
	0		$og L_{max}$
	β		1,754
	β and γ	-18	
	$\alpha$ , $\gamma$ , $\alpha$ and $\gamma$		3.625
	Support for $\beta$ relative to log I	$L_{max} = -181.75$	4
	Units of support	Value	s of β
		Upper	Lower
		limit	limit
	3 units	0.692	0.097
	2 units	0.660	0.184
	I unit	0.613	0.283
ь.	Arctic skuas of Foula		
•	Parameters estimated	Maximum lo	g likelihood
	a		3.028
	α and β		2.364
	$\beta$ , $\gamma$ , $\beta$ and $\gamma$	28	
	Support for a relative to log I	$L_{\rm max} = -283.02$	
	Units of support	Value	
	Omits of support		
		Upper	Lower
	2	limit	limit
	3 units	0.523	0.069
	2 units	0.488	0.117
	l unit	0.441	0.177

The levels of support are units of log likelihood below the maximum log likelihood. For example, for the Fair Isle skuas, the 2-unit support limits are the values of  $\beta$  for which log L=-183.754, being two units of log likelihood below the maximum when the one parameter, β, is estimated. If variation is normally distributed, the 2-unit limits correspond to the limits  $\pm 2\sigma$ , where  $\sigma$  is the standard error of the estimate. The variation in these estimates, is however, not normal and the limits are not symmetrical, although the difference between the upper and lower 2-unit limits is approximately 40. The difference between these limits would be exactly 40 if the variation were normal.

O'Donald<sup>5</sup> showed that at equilibrium the frequency of the heterozygotes is given by the solution of the equation

$$[(1-\frac{1}{2}\beta)(\frac{1}{2}\alpha-\beta+\frac{1}{2}\gamma)-\frac{1}{2}(\alpha-\beta)(\gamma-\beta)]v^2+[(1-\frac{1}{2}\beta)(1-\alpha p-\gamma q)-q(\alpha-\beta)(1-\gamma)-p(\gamma-\beta)(1-\alpha)]v-2pq(1-\alpha)(1-\gamma)=0$$

where p and q are the gene frequencies and v is the frequency of the heterozygotes. To apply this formula to the Arctic skuas, we must assume that the intermediates are the heterozygotes6. There is in fact a continuous range of variation from intermediates to completely dark birds. In our present work we have classified as intermediate any bird with at least some paler feathers on the nape. Dark birds seem to be homozygous by this classification. Using our estimates of the parameters, we predict that the frequencies of heterozygotes at equilibrium should be 0.4748 on Fair Isle and 0.4563 on Foula, compared with the actual frequencies of 0.5905 and 0.4606 respectively. The predicted frequency is very close to the actual frequency on Foula, but very significantly different from it on Fair Isle. Indeed, on Fair Isle, there is a significant excess of intermediates over the numbers of heterozygotes expected by random mating. This may indicate that natural selection favours heterozygotes on Fair Isle or that differential migration has occurred and equilibrium has not yet been reached.

Assortative mating differs from sexual selection only to the extent that the mating preferences are restricted to individuals of the same phenotype as that which is preferred. Assortative mating and sexual selection will have similar selective effects as a result of female mating preferences: the preferred males will gain an advantage because they will mate earlier in the breeding season. The preferences have been estimated from the Fair Isle data of breeding dates of pale, intermediate and dark males3. In the model with the highest likelihood, 18% of the females prefer dark males and 29% prefer intermediate males. Using our present data, we have estimated that 47% of the matings of intermediates are assortative. These matings are probably caused by the preference of intermediate females

for intermediate males. The 47% of these females represents 29% of all females—almost exactly the estimate of all females who prefer intermediates.

Intermediate females breed on about the same average date as intermediate males. This is to be expected as a result of the assortative mating. The similar breeding dates of the other females show that the preference for dark males, which are the first to breed, is distributed at random among the female phenotypes, giving rise to sexual selection for the darks without assortative mating.

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## **Cascading speciation**

CENTRAL to the theory of allopatric speciation is the idea that geographic separation of populations will sufficiently restrict gene flow between them to make possible their genetic divergence1. Wright2 has shown that, at equilibrium, the arrival of one migrant on average every other generation is sufficient to keep a population's genetic composition similar to the source of migrants—at least when selection is not too strong. Wright's result has been extended to one- and two-dimensional arrays by Maruyama3.

There may be some difficulty in describing the usual course of the speciation process in the light of these theoretical results. If one or several dispersing individuals from an established population can reach some location and start a new population, then what is to prevent others arriving from the same source and preventing local differentiation? One possible answer is that speciation depends entirely on infrequent and accidental events. For example, small and large scale geological changes can completely prevent gene flow that was once possible or the 'founder effect'1 could produce rapid enough genetic changes in a newly-founded population that some degree of reproductive isolation could evolve before many more migrants arrive. It is also possible that many species evolve from migrants which have gone sufficiently far from their parent colony that the arrival rate of other migrants from the same colony is far below Wright's limit.

Another mechanism for species formation and one which does not require accidental events is disruptive selection resulting from the ecological conditions in different microhabitats. Experiments have shown that reproductive isolation can sometimes evolve in the presence of gene flow and strong disruptive selection, and theoretical models<sup>6</sup> reinforce that conclusion. Disruptive selection, however, cannot account for the abundance of sibling species or closely related species which are similar in geographic distribution and ecological requirements<sup>1</sup>.

All these factors have been important in evolution, and it is possible that they are sufficient to account for the known rates of speciation. I propose, however, that if the non-equilibrium properties of genetic systems are considered, then the existence of accidental processes or disruptive selection is not necessary for all cases of speciation. In particular, there is often no need to assume that there is some mechanism which 'shuts the door' on future migrants so that speciation may occur.

My argument follows from well known theoretical results. For a diploid species, consider a population of size N which receives m migrant individuals from a second population, and consider a locus segregating for two alleles A and a. Suppose that A is fixed in the second population and a in the first. Then in each generation in which a migrant arrives at the first population, the probability of ultimate fixation (u) of either of the arriving alleles is

$$u = [1 - \exp(-4s)]/[1 - \exp(-4Ns)] \tag{1}$$

where s is the selective advantage of A over a (assuming no dominance and independence of the alleles?). For  $Ns \ll 1$ , u is approximately 1/N and for  $Ns \gg 1$ , approximately 4s. If we assume that each migrant is an independent trial, then the expected time until the arrival of the A allele which will ultimately be fixed is 1/mu. The expected time until fixation of the successful allele must be added to its expected arrival time8. Thus, there is a time lag in the response of a population to gene flow. In this example, if m = 1 and s = 0.01 the time lag until fixation begins (which is a minimum estimate of its importance) is 25 generations. With s = 0.001, the lag increases to 250 generations.

If instead we assume A is increasing deterministically in the second population according to

$$p(t) = \left[ 1 + \frac{(1 - p_0)}{p_0} \exp(-st) \right]$$
 (2)

where  $p_0$  is its initial frequency<sup>4</sup>, then the average number of A alleles carried by each migrant is 2p(t). The probability of ultimate fixation is given approximately by equation (1) so the expected time until fixation of A begins in the first population is

$$\int_0^\infty tump(t) \exp\left[-\int_0^t ump(t)' dt'\right] dt$$

$$= \int_0^\infty \exp\left[-\int_0^t ump(t') dt'\right] dt \tag{3}$$

where the second expression is obtained by integrating by parts. With m = 1,  $p_0 = 0.001$  and s = 0.01, the time lag is 420 generations and with s = 0.001, 4,200 generations. Before A is established another allele serving the same purpose could arise and become fixed. In this way genetic differences could accumulate.

With only two populations, each of which is relatively permanent, the time lag effect may not lead to the evolution of genetic differences, as it requires that new alleles with similar functions arise within a relatively short time. If there were a large number of populations in an array, however, then the time lag would increase roughly linearly with the number of steps separating two populations, and consequently could be significant for populations at opposite ends of the array. The wave of advance of an advantageous allele, which was modelled. by Fisher<sup>9</sup>, would be considerably slowed.

The time-lag effect could also be important when the expected time until extinction of a population is of the same order of magnitude as the time lag. If there were a collection of populations, each of which had some probability of providing the propagules to found new colonies and each of which went extinct at a certain rate, then genetic differences could develop

in different populations as a result of independent mutations in each. The time lag effect would prevent swamping by gene flow between the populations. Such a process could account for the genetic differences between populations of Mus musculus10 taken from a single barn. Those differences would be difficult to explain using an equilibrium theory.

The accumulation of small genetic differences by the combination of the time lag effect and the extinction and colonisation of local populations can be thought of as a cascading process in which the new populations which are founded are likely to be slightly different from the average of the collection of populations. The differences can result both from selection and genetic drift. The accumulation of differences can lead to speciation. I will call this process 'cascading speciation' to distinguish it from speciation processes in which populations are approximately in equilibrium. Cascading speciation skill depends on accidental events, namely extinction and recolonisation, but ecological studies have shown that extinctions and colonisations are relatively frequent events in many species11, and can take place on the same time scale as the time lag associated with gene flow.

If cascading speciation has been an important process, then one consequence is that speciation rates within taxonomic groups with many fugitive species12, which presumably have higher extinction rates and greater dispersal ability, would be higher. If equilibrium processes have been more important, then in such groups speciation rates would be lower because of the higher level of gene flow resulting from the greater dispersal tendencies. One possible example of this is the Drosophila of Hawaii<sup>13</sup> which contains a large number of species and for which there is assumed to be a high rate of extinction of local populations<sup>14</sup>. Carson<sup>14</sup>, in discussing the Hawaiian *Drosophila*, has emphasised the importance of inbreeding in small local populations which would enhance the founder effect and the role of genetic drift; but that does not change the limit on gene flow imposed by the equilibrium theory, which is independent of local effective population size.

In conclusion, I should emphasise that my point is quantitative, not qualitative. Geographical isolation is still necessary for speciation, but if the extinction of local populations is considered, then the degree of isolation necessary is not as great as is often assumed.

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## Isolation by distance in Liatris cylindracea

Several factors may cause genetic correlation—that is a genetic relationship between individuals within a populationincluding small population size, self-fertilisation, positive assortative mating or isolation by distance. Isolation by distance occurs when gene dispersal is limited so that distant populations in a series or remote areas within a population become genetically differentiated. Under an isolation-bydistance model of genetic differentiation, spatial distance and genetic correlation between individuals or populations are expected to be negatively related, that is, the greater the distance the smaller the genetic correlation. I wish to present evidence for isolation by distance in the plant, Liatris cylindracea Michx. (Compositae), obtained by a genetic distance analysis of gene frequencies at 27 allozyme loci. This analysis revealed genetic differentiation across very small distances and demonstrated that a restriction of gene flow has a profound effect on the spatial distribution of alleles within a population.

Plant species are prime candidates for the study of isolation by distance since they are immobile and gene dispersal in both the pollen and seed phases is often quite restricted. Indeed the earliest study of isolation by distance was on the annual herb Linanthus parryae, which shows genetic differentiation for flower colour. Liatris cylindracea, a prairie gayfeather, is a perennial, obligately outbreeding herb which grows in undisturbed prairies throughout the mid-west of the United States. A single, dense population<sup>2</sup> on a hillside in Zion, Illinois was divided into a grid 18×33 with 3 m<sup>2</sup> quadrats. Up to 60 plants were collected from each quadrat; the collection totalled 2,258 individuals. Starch gel electrophoresis was performed on the plants for 12 different enzyme systems, encoding 27 genetic loci. Gene frequencies were calculated on a per quadrat basis.

Evidence for isolation by distance was provided by analysis of genetic distance within the Zion population. Genetic distance is an index for measuring the accumulated number of gene differences per locus between groups of organisms. Of several available measures of genetic distance I used Nei's because it uses allozyme gene frequency data and, most importantly for a study of isolation by distance, it is related directly to Malecot's coefficient of kinship,  $\varphi$ . Nei's genetic distance is defined as:

$$D = -\log_e(J_{xy}/\sqrt{(J_xJ_y)})$$

where  $J_x$ ,  $J_y$ , and  $J_{xy}$  are the arithmetic means of the probabilities of two randomly chosen genes being identical in populations x, y, and x and y. Genetic distance is expressed as codon differences per locus.

Genetic distance in the L. cylindracea population was calculated for 103 quadrat pairs which had data from all 27

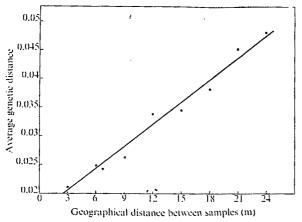


Fig. 1 Genetic distance in L. cylindracea. The genetic distance plotted is the average of all quadrat pairs for a given spatial distance. Geographical distance is the distance (m) between sample quadrats.

allozyme loci. Measurements of genetic distance between sample quadrats showed considerable variation. The mean genetic distance averaged from all of the distances computed is  $0.0396 \pm 0.004$  codon differences per locus. Genetic distance between quadrat pairs ranged from 0.0061 to 0.1156.

Considerable local differentiation within the population is indicated by the genetic distance measures. If genetic differentiation is the result of restricted migration, genetic distance would be expected to be related to spatial distance. The average genetic distance measured between sample quadrats is plotted against spatial distance in Fig. 1. As spatial distance increases between sample quadrats, the genetic distance increases proportionately. This linear relationship of spatial and genetic distance suggests that isolation by distance has occurred in the Zion population.

These results are consistent with the population biology of L. cylindracea. Both phases of gene dispersal, pollen and seed, are very restricted4. Pollen is transferred mainly by bees; field observations on L. cylindracea indicate that most moves by pollinators are to the nearest neighbour. Likewise seed dispersal, although by wind, is limited. This restricted gene dispersal is manifested in the spatial variation of gene frequencies within the population. Statistically significant gene frequency differences of as much as 0.2 existed between neighbouring quadrats. Genetic variation at a single locus, however, generally does not correlate to environmental parameters within the population<sup>2</sup>. Genetic divergence over distance appears to be a function of random gene frequency fluctuations. Only when all loci are considered together does the pattern of genetic variation in the Zion population emerge; genetic distance is positively related to spatial distance. This relationship is as predicted for a population in which gene dispersal is limited and genetic differentiation results from isolation by distance.

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# Implications of ethylene production by bacteria for biological balance of soil

ETHYLENE in soil causes fungistasis<sup>1</sup>, affects the growth of bacteria, actinomycetes and nematodes (unpublished data) and consequently must influence many soil biological processes. *Mucor hiemalis* has been proposed as a major producer of ethylene in soil<sup>2</sup> although earlier work suggested that facultative anaerobes may be involved<sup>3</sup>. Our studies show clearly that the main production of ethylene in soil is by spore-forming bacteria in anaerobic microsites. These bacteria seem to be an integral part of a self-regulating cycle in soil controlling microbial activity and with far-reaching implications for the biological balance of soil.

A high organic matter, basaltic soil (Ashburner!), which produces high levels of ethylene, was treated moist for 30 min with aerated steam at 60°, 82° and 100° C to simplify progressively the soil microflora. Samples of treated soil were compared for ethylene production with untreated and autoclaved (60 min at 121° C) samples. Untreated and treated soil samples were dried aseptically at 37° C for 24 h, passed through a 0.2-cm mesh sieve, placed as 5-g lots in four replicate sterile glass vials (14 ml capacity), wetted to 40% water (-0.5 bar water potential) with sterile distilled water, sealed with rubber septa and incubated at 25° C. This is our standardised assess-

ment method. Production of ethylene was reduced significantly only in soil heated above 82° C (Fig. 1). Small quantities of ethylene were detected following treatment at 100° C, but virtually none formed in autoclaved soil. Spore-forming bacteria (both aerobic and anaerobic) were the only significant survivors in soil treated at 60° C and above; some survived the treatment at 100° C, but none survived 121° C. Similar results were obtained with several other soils after treatment with aerated steam. In another test, soil slurries in tubes were heated at 80° C for 1 h in a water bath and then compared for ability to produce ethylene with non-treated slurries when incubated at 25° C. The treatment enhanced ethylene production, especially initially, probably because of heat activation of spores<sup>5</sup>. These results rule out *Mucor hiemalis* as a major producer of ethylene in soil² because it is killed by aerated steam at 60° C or above¹.

In another series of experiments, Ashburner soil was wetted to different water potentials; ethylene was produced at -5 bar and wetter, but only slightly or not at all at -15 bar or drier. Soil bacteria, but not actinomycetes or fungi, are limited by water potentials below -15 bar<sup>6</sup>.

Production of ethylene under aerobic as compared with anaerobic conditions was studied with Ashburner soil wetted to 40% water and incubated at 25° C in the vials under atmospheres of air, nitrogen, argon or hydrogen. The atmospheres in vials of dry soil were evacuated twice and replaced each time with the desired atmosphere. Oxygen-free water was then added by syringe to the soil surface and allowed to move as a wetting front to the bottom of each vial. In all tests, ethylene production was greatest in the absence of oxygen (Fig. 2). A similar result was obtained with a cultivated garden soil (Penrith¹) which normally produced little ethylene. Additionally, ethylene production under a nitrogen atmosphere in Ashburner soil was prevented, or nearly so, by a water potential of—15 bar, confirming the role of anaerobic bacteria.

Amendment of soils with different forms of nitrogen markedly affected ethylene production. Nitrate always delayed production in proportion to the amount added whereas ammonium stimulated production, or had no effect, depending on the soil used. Ethylene production was best in soils leached free of nitrate but if such soils were reamended with nitrate at 20–200 p.p.m. N, production started only after a period of denitrification, or not at all. Nitrate acts as a terminal electron acceptor in the absence of oxygen and can poise the redox potential of soil sufficiently to prevent activity of strict anaerobes<sup>7,8</sup>. This seems to explain how the presence of nitrate prevents ethylene production and indicates that production occurs only under highly reduced conditions in soil.

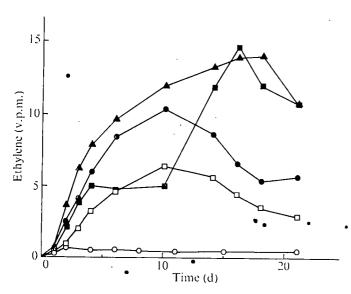
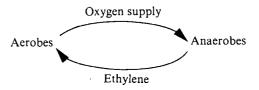


Fig. 1 Ethylene production by Ashburner soil following heat treatment. ♠, Untreated; ♠, aerated steam for 30 min at 60° C; ♠, 82° C; ☐, 100° C; ○, and autoclaved for 60 min at 121° C.

Although ethylene production was best in oxygen-free atmospheres, it was consistently formed, sometimes at high levels, in soil incubated under air and as dry as -5 bar water potential. It was also detected *in situ* at adjacent sites in a soil under mown grass (about 0.5–6.0 p.p.m.) and cultivated (about 0.03–0.5 p.p.m.), including when the soil was considerably drier than field capacity and hence probably well-aerated.

Any apparent conflict in the presence of ethylene in soil under the conditions described and its anaerobic requirements for production, are explained by the occurrence of anaerobic microsites in even well-drained field soil. These anaerobic microsites are continually formed in soil as a result of aerobic microorganisms utilising oxygen especially near organic matter9. Anaerobes will proliferate in these microsites and produce ethylene which diffuses through the soil. Sensitive species of aerobic microorganisms will be inactivated first, but eventually if levels become high enough, most, if .not all, aerobic microbial growth probably will be arrested by the ethylene. The system then becomes self-regulatory because as oxygen demand falls, oxygen will diffuse back into the microsites and prevent anaerobic ethylene production; this will permit resumption of aerobic growth. We also have evidence that the anaerobes depend, at least in part, on aerobes for production of substrate for ethylene formation. This cycle may be summar-



Verification of such a cycle comes from numerous observations made during the glass vial tests on ethylene production in soil. Prolific hyphal growth was observed through the glass vials under a dissecting microscope on the moist soil crumbs when ethylene was low but not when ethylene was high. In tests allowed to continue for over 80 d, ethylene levels reached a peak, dropped, then eventually rose again in cyclic fashion; fungal hyphae appeared, disappeared (lysed) and reappeared in response to the fluctuations. Actinomycetes behaved similarly except that colonies became evident only at intermediate levels of ethylene and after most fungal hyphae had lysed. At high levels of ethylene these colonies also disappeared.

Large inputs of nutrient temporarily override the suppressive effects of ethylene possibly in much the same way fungistasis is annulled by nutrients. Without this means of adjustment, decomposition of organic matter would be slowed, with consequent problems of accumulation in soil.

The concept of an oxygen-ethylene cycle assigns an important microbiological role to the ubiquitous spore-forming anaerobes of soil; by producing ethylene, they prevent microbiologically active soils from becoming predominantly anaerobic. This ensures that the more efficient aerobic respiration dominates the soil system. Anaerobic production of ethylene also explains why ethylene producers do not dominate a soil—anaerobes are prisoners of their microsites.

In our studies, bare cultivated soil has always produced less ethylene than undisturbed or cropped soil regardless of whether measured *in situ* or removed, dried, sieved and tested in the standardised vial test in the laboratory. This has important implications for agriculture because these results indicate that the oxygen-ethylene cycle is in balance in soil under undisturbed ecosystems such as grasslands and forests, where organic matter and plant nutrients are slowly recycled, nitrate nitrogen does not accumulate, and root diseases are rare. Conversion to agricultural usage destroys the balance because tillage aerates the soil, elevates the redox potential and eliminates anaerobic microsites. This increases organic matter breakdown

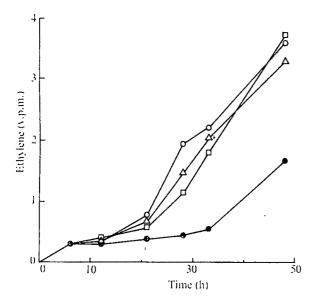


Fig. 2 Ethylene production by Ashburner soil under atmospheres of ●, air; ○, nitrogen; △, argon; □, and hydrogen.

and release of nitrogen. Plants are not present to use the ammonium nitrogen which is rapidly nitrified. Even if oxygenfree microsites redevelop, ethylene production will not resume until nitrate has been removed by plant roots of a subsequent crop, or less desirably, by leaching or denitrification. This seems to explain adequately why cultivation causes an inevitable decline in levels of organic matter, soil structure, and nitrogen availability and an increase in root diseases. Also, the presence of nitrate causes a loss of cations from soil<sup>10</sup> and a paucity of reduced microsites which affects the availability of iron, manganese and phosphorus8.

Precise manipulation of the oxygen-ethylene cycle in soil offers a means of regulating energy turnover and availability of plant nutrients as well as improved control of soil-borne plant pathogens. These results call for a reappraisal of the value of nitrate in agriculture and give new justification for minimum tillage and the use of organic amendments in farming.

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## Pigments responsible for ultraviolet patterns in flowers of *Oenothera* (Onagraceae)

Many insect-pollinated flowers have nectar guides1 which increase the foraging and pollination efficiency of their insect visitors2. Some of these are visible only by their contrasting ultraviolet patterns, visible to insects but not to humans3. In such flowers, the nectar guides absorb ultraviolet light, and the compounds responsible were early suspected to be phenolics4. Recently, in Rudbeckia hirta L. (Asteraceae), a mixture of methylated flavonol glycosides has been shown to be responsible5. We report here that another class of flavonoids, chalcones, is responsible for the similar patterns in Oenothera (Fig. 1) and in three other genera of Onagraceae with flowers that appear uniformly yellow to humans.

Fresh petal material of Oenothera hookeri Torr. & Gray ssp. venusta (Bartlett) Munz was divided into ultraviolet-reflective and ultraviolet-absorptive portions. Because these areas cannot be distinguished visually, only small portions of the base and apex of the petal were used to minimise contamination. The material was extracted with methanol for several days to remove visible as well as ultraviolet-absorbing material. Extracts were analysed spectrally and chromatographically.

The absorption spectrum of the extract from the apical reflective portion of the petal had maxima at 460 nm, 435 nm and 415 nm (characteristic of carotenoids), a second maximum at 265 nm (probably non-flavonoid phenolic compounds) and a minimum between 310 nm and 370 nm (Fig. 2A). Extracts from the basal, absorptive portion of the petal had an additional strong maximum at 365 nm (Fig. 2A).

The two-dimensional paper chromatogram of the apical extract indicated that only carotenoid was present. The basal extract contained carotenoid plus an ultraviolet-absorbing pigment which was identified as the chalcone glucoside isosalipurposide<sup>6</sup>. The absorption spectrum of a methanol solution



Fig. 1 Fresh flower of Oenothera grandis in daylight (top) and in ultraviolet light (366 nm) with a Kodak Wratten 18A filter (bottom).

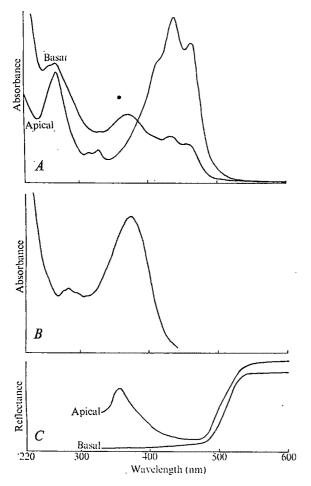


Fig. 2 A, Absorption spectra of methanol extracts of portions of petals from Oenothera hookeri. B, Absorption spectrum of a methanol solution of the chalcone, isosalipurposide. C, Reflectance spectra of portions of a petal of O. hookeri.

of this compound had a single absorption maximum at 365 nm (Fig. 2B). Thus the restricted distribution of the chalcone in the basal portion of the petals results in the observed differences in light reflection. The reflectance spectra of the two portions of the petal (Fig. 2C) also support this conclusion.

The internal anatomy of the petal suggests that the mesophyll is a diffusing and pigmented structure with transparent plates composed of epidermis on both surfaces. Thus much of the light striking a petal is reflected unless it is absorbed by pigment molecules in the mesophyll. In the case of the basal portion of Oenothera petals, the presence of flavonoids absorbing in the near ultraviolet and carotenoids absorbing between 400 and 470 nm results in low reflection of wavelengths 330-480 nm (ultraviolet and blue light) with high reflection in the yellow region of the spectrum giving rise to yellow coloration for both humans and insects. The apical portion of the petal containing only carotenoid reflects ultraviolet as well as yellow light. Thus it appears yellow to humans but bee purple (a combination of ultraviolet and yellow light analogous to human purple which we distinguish when light from both ends of our spectral sensitivities, red and blue, are mixed) to an insect.

The observation of fluorescent patterns under ultraviolet light in pressed herbarium specimens matching the invisible ultraviolet patterns in fresh flowers8 fits this model of petal pigmentation. Flavonoids are stable while carotenoids are liable to autoxidation in air<sup>9,10</sup>. The breakdown products of carotenoids presumably fluoresce under ultraviolet light, whereas the stable flavonoids continue to absorb. Flavonoids commonly appear as dark spots on paper chromatograms illuminated with ultraviolet light.

Other Oenothera species with yellow flowers which absorb

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ultraviolet light were examined chromatographically. These represent not only the closely related subgenera Oenothera (O. elata HBK., O. maysillesii Nutt. ex Torr. & Gray) and Raimannia (O. drummondii Hook., O. grandis (Britton) Smith, O. rhombipetala Nutt. ex Torr. & Gray) but also the more distantly related Hartmannia (O. epilobiifolia HBK., O. seifrizii Munz). Results were similar to those for O. hookeri, showing carotenoids present throughout the petal and the chalcone restricted to the absorptive areas. Another group of flavonoids, quercetin derivatives, was found in addition to the chalcone in the basal portions of the petals of all species, although not as abundantly in O. grandis and O. rhombipetala. Myricetin derivatives were also absent from petals of subgenera of Oenothera and Raimannia and present in those of Hartmannia, as reported for their leaves<sup>11,12</sup>. Like the chalcone, myricetin derivatives have absorption maxima in the near ultraviolet.

With the results presented here, two classes of flavonoids, flavonols (in Asteraceae)5 and chalcones (in Onagraceae), have been found associated with absorption of ultraviolet light in nectar guides, indicating that these compounds together with anthocyanins and carotenoids are important in flower pigmentation. The chalcone isosalipurposide plays a role similar to that demonstrated here in Oenothera in other yellow-flowered Onagraceae belonging to the genera Camissonia, Gaura and Ludwigia6.

The involvement of flavonoids in these pigment systems important in plant-pollinator interactions suggests a role for the flavonoids which are ubiquitous in the leaves of angiosperms and contribute to absorption in the ultraviolet portion of the spectrum. Leaves reflect a low level of light with spectral quality such that insects perceive it as grey, a neutral colour3. This means that plants which depend on insect visitors for pollination can advertise their flowers as brightly coloured entities against a dull, neutral background and thus increase the efficiency of their pollinators.

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#### Tumbling in pigeons

THE spectacular hereditary trait of backward somersaulting by Tumbler pigeons has been known since 1600 AD1 and was described by Darwin as ". . . one of the most remarkable inherited habits or instincts ever recorded...". The potentially important physiological mechanism of tumbling is unknown, largely because the extreme rapidity of the somersaulting blurs visual observation of the component motions. Filming at 2,000 frames per second the normal flight of Racing Homer pigeons and characteristic tumbling episodes by Parlor (nonflying) Tumbler pigeons, we observed the following. The body position of a pigeon just before and during tumbling is associated with abnormal dorsiflexion of the tail, which occurs within 15 ms after the pigeon is released. Tumbling is synchronised with apparently normal wing movements, propelling the pigeon in only a backward direction, at a rate of 8–10 somersaults per second.

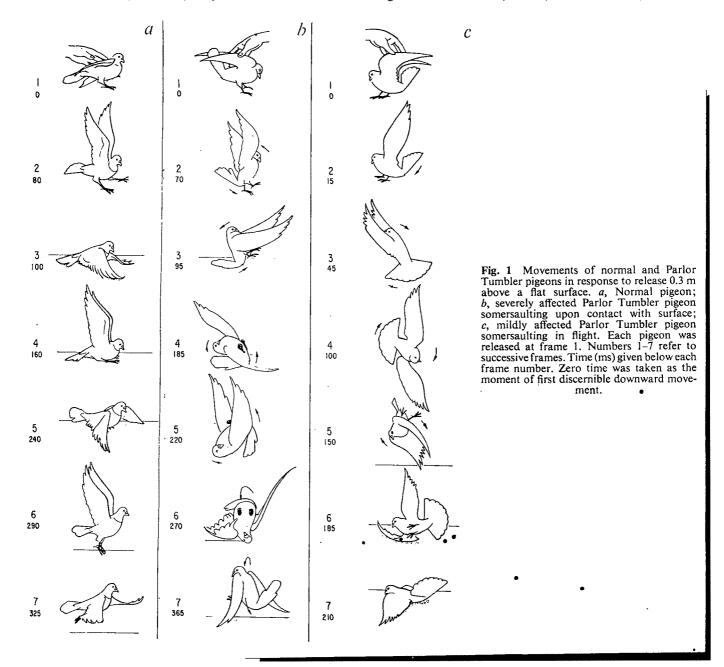
Each filming session involved releasing a hand-held pigeon 0.3 m above a flat surface and activating the camera and high intensity lamps for a period of 1-2 s. Although we were mainly interested in the initial tumbling motions, 5-10 somersaults by each pigeon were filmed during this short time. The film was analysed frame-by-frame and at 16 frames s<sup>-1</sup>. Sketches from representative frames with corresponding times (ms) are shown in Fig. 1.

Figure 1a shows that the normal pigeon raises the wings and extends the feet upon release and lands on the feet with wings forward. In addition, the head, body and tail remain in line

and parallel to the surface below during the descent. Frames 4–7 in Fig. 1a illustrate the initial flying motions, consisting of phasic wing beats accompanied by a forward shift of the body mass that result in flying less than 200 ms later. As in the descent, the head, body and tail remain in line as the pigeon ascends.

Pigeon fanciers and researchers alike use number of somersaults per unit time, duration of a tumbling episode and ease of provocation of tumbling to categorise Tumbler pigeons<sup>3-7</sup>. A severely affected bird will begin somersaulting with each mild provocation such as approaching the bird or snapping the fingers. Less severely affected birds sometimes simply run from the investigator and may somersault only in response to a loud hand clap or to dropping from a height.

The pigeon depicted in Fig. 1b is a severely affected Parlor Tumbler, while that in Fig. 1c represents a milder form. The severely affected Tumbler raises the wings and extends the feet upon release (frames 1 and 2) as does the normal pigeon (Fig. 1a) but the head and body of the Tumbler are not parallel to the surface below. Also the tail of the Tumbler forms an acute angle with the rest of the body. The tail elevation in the Tumbler occurs within 15 ms of release (not shown) and the tail remains in this abnormal position throughout the tumbling episode. The milder form (Fig. 1c) lands with feet and wings extended forward (frame 3) but neither wings nor feet



contact the surface as in the normal pigeon. The abnormal landing appears to be related to the backward angular rotation (nose-up pitching) during descent. Both wings and tail may be involved in the rotatory movement, but we suggest that the tail is the main influence. The initiation of the somersault is seen in Fig. 1b (frame 3) where the pigeon has started to pitch as the wings move forward. Subsequent wing movements appear appropriate for flight, but the abnormal posture maintained during tumbling causes the wing beating to propel the pigeon backwards. A cycle of a single wing beat in the normal pigeon and severely affected Tumbler b lasts 100-125 ms; the head and body remain aligned in both, but the tail is elevated only in the Tumbler. Subsequent somersaults are stereotyped and occur at a rate of 8-10 per second with one wing beat per somersault.

Figure 1b also shows that movements other than pitching rotation occur during tumbling. These are rotation about the longitudinal body axis (rolling) and rotation about a dorsoventral body axis (yawing) which cause the pigeon to land in a position different from that expected after a simple 360° pitching motion. Comparison of frames 3 and 7 in Fig. 1b illustrates the effect of these superimposed motions.

Figure 1c shows tumbling in flight. Wings and feet are again extended and the tail elevated within 15 ms after release (frame 2). After 45 ms descent the pitching motion has positioned the pigeon abnormally but head and body remain aligned (frame 3). The propulsive force for tumbling in flight, as on the ground, is apparently supplied by the wings. The first evidence of tumbling occurs in frame 3 as the forward wing stroke begins; 140 ms later the episode ends in an awkward landing as the cycle is completed (frame 6). Frames 4 and 5 illustrate the rolling and yawing motions, and their influence on the pigeon's ultimate position.

All the Tumbler pigeons we observed had apparently normal wing motions. The force was supplied by the forward wing stroke during which the feathers appeared tightly interlocked. During the back stroke the wing feathers separated and sliced through the air with little resistance. Furthermore, the position of the wings relative to the body was the same in normal and Tumbler pigeons. The feet did not seem to contribute to tumbling; their position varied from one somersault to the next and no pushing movements were observed. In fact, in some cases the feet seemed to decrease the tendency to tumble by exerting a dragging force opposite in direction to the characteristic pitching motion.

The stereotyped abnormality of the Tumbler pigeon possibly arises from the abnormal function of a sensory input, a central or feedback mechanism, or an aspect of the motor output.

The fact that the abnormal response occurs within 15 ms of the pigeon being released is consistent with neural transmission of a stereotyped movement. The inner ears of Tumbler pigeons are normal<sup>6,7</sup>, the cerebella appear normal by light microscopy (R.K.E., and S.H.B., unpublished observation) and earlier experiments indicate that tumbling is not a form of epilepsy<sup>5,6</sup>. In addition, we have recently reported that skeletal muscles of Tumbler pigeons are not myotonic<sup>8-9</sup>. Experiments in progress suggest that tumbling is associated with an abnormality of the central nervous system. Irrespective of the mechanism of tumbling, a better understanding of motor control in animals with prominent basal nuclei might be gained from further studies of Tumbler pigeons.

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## Changes in heroin self-administration by a rhesus monkey after morphine immunisation

We have investigated in one primate the effect of immunisation against an opiate on the self-administration of these drugs. Using the rhesus monkey, which will self-administer various drugs, including opiates and psychomotor stimulants1, we have followed the response for heroin before and after immunisation against morphine. Results indicate that antibodies against morphine can block those effects of heroin on the central nervous system (CNS) that maintain self-administration behaviour.

Our procedure was based on existing knowledge of the immune response against opiates. Antibody against morphineprotein conjugates has been demonstrated to bind free opiate hapten2-8. Different antibody specificities have been described when the same immunising opiate was linked to the protein carrier through different positions on the chemical nucleus<sup>3.5-8</sup>. Antiserum obtained after immunisation with morphine-6hemisuccinvl-bovine serum albumin (M-6-HS-BSA), the conjugate we used, has highest and approximately equal affinity for morphine and heroin and progressively less for opioids of decreasing structural similarity.

In vitro studies based on the observation that morphine inhibits electrically stimulated contractions of isolated guinea pig ileum longitudinal muscle have shown that anti-M-6-HS-BSA globulin can block a peripheral pharmacological effect of morphine<sup>9,10</sup>. Concentrated antibody was found to reverse the inhibition of muscle contractions caused by 120 nM morphine in a manner apparently identical to that observed after addition of 10 nM of the narcotic antagonist naloxone11. The drug self-administration model enables extension of these studies to CNS actions of opiates in vivo.

The apparatus used was a modification of that illustrated by Schuster and Johanson<sup>1</sup>. A double lumen polyvinyl chloride catheter was implanted into the right internal jugular vein of a 5.6 kg rhesus monkey and passed subcutaneously to an exit point on the animal's back.

While self-administering drug, the monkey was housed in a wooden cubicle and minimally restrained by a stainless steel harness and attached hollow steel spring arm. The catheter extended from its exit on the monkey's back through the arm, out the back of the cubicle and through a peristaltic infusion pump to connect with a drip bag reservoir of drug solution or saline. The cubicle was sound-attenuated and approximately 75 cm  $\times$  75 cm  $\times$  90 cm. The front door held an observation window, ventilation fan and two boxes, each with a lever and stimulus light. This allowed separate levers and stimulus lights to be associated with heroin and cocaine. A third stimulus light was located high on a side wall.

The monkey was trained to self-administer heroin and cocaine in aqueous solutions of 0.9 % NaCl on a fixed ratio ten schedule (ten presses of the correct lever required for delivery of one infusion). Cocaine, which is not significantly bound by antiM-6-HS-BSA, was used to detect nonspecific changes in drug taking behaviour after immunisation. Such changes could conceivably result from toxic effects of the immunising preparation or from the lack of opportunity to self-administer drug during immunisation, although trained animals after periods of several weeks without access to drugs will readily resume self-administration (unpublished results of C.R.S.). Only one of the two drugs was available each day for a 2-h session, and the sequence of drug availability was varied. The appearance of a stimulus light overhead and another on the appropriate lever signalled the start of each drug session. During all infusions the lever light was turned off and the colour of the overhead light changed. Stimulus lights were controlled and lever presses and infusions taken were recorded by electromechanical programming equipment located in an adjoining room.

Infusion volume was 0.2 ml kg<sup>-1</sup> delivered at approximately 6 ml min<sup>-1</sup>. The heroin dose was 6 μg per kg per infusion, chosen to maintain self-administration behaviour without producing physical dependence that would complicate the two-drug baseline. The number of infusions taken per session for each drug was approximately equated by adjusting the cocaine dose, resulting in 100 µg per kg per infusion of cocaine.

After heroin and cocaine intake had stabilised, substitution

of normal saline demonstrated that the self-administration of heroin and cocaine were independent of each other and that the monkey could detect the absence of either drug in spite of the presence of the usual stimuli associated with drug reinforcement. Two such heroin extinctions and one cocaine extinction observed before beginning immunisation are shown in Fig. 1. The first extinction for each drug showed a rapid drop to zero infusions self-administered per session. The second heroin extinction showed a more typical pattern, an initial increase in responding and number of infusions taken, followed by a drop to an infusion total per session well below the drug baseline but above zero12. When drug solution was reintroduced in each case there was a prompt return to self-administration behaviour. During the saline infusions, intake of the unsubstituted drug varied only within the usual baseline fluctuations. The stability of heroin intake per session throughout this 2-month period also indicates that tolerance was not a significant factor regulating self-administration.

Drug sessions were discontinued and the monkey was transferred to a metal grid cage. He was then immunised subcutaneously with 5 mg M-6-HS-BSA conjugate in complete



Fig. 1 Effect of substitution of saline for drug solutions on selfadministration by a rhesus monkey. Total infusions taken per 2-h session are shown. 

Heroin 6 µg per kg per infusion; 
 ¬, saline infusions accompanied by heroin-associated discriminative stimuli; 
 ¬, cocaine 100 µg per kg per infusion; 
 ¬, saline infusions with cocaine-associated discriminative stimuli.

Freund's adjuvant. A second 5-mg injection was given 28 d later. Every 2 weeks thereafter 10 mg of the conjugate in incomplete Freund's adjuvant was given. After 20 weeks the serum morphine-binding capacity at 100 pmol ml<sup>-1</sup> <sup>14</sup>C-morphine added was 77,550 pmol ml<sup>-1</sup> undiluted antiserum<sup>8</sup>. Radioimmunoelectrophoresis demonstrated that the morphine was specifically bound to the IgG fraction of serum from the monkey. (We have also found high levels of morphine binding in antisera from two other monkeys immunised with M-6-HS-BSA.)

A catheter was implanted in the left internal jugular vein and the animal, now weighing 6.6 kg, was returned to drug selfadministration. The monkey resumed his former baseline level of cocaine intake but failed to respond for heroin with a frequency significantly above that for saline (Fig. 2). The heroin dose was then successively doubled, allowing a minimum of three heroin sessions per dose. Self-administration was reinitiated at 100 µg per kg per infusion with an average of 16 infusions taken per 2-h session. This is approximately 16 times greater than the original reinforcing dose per infusion and a concentration too depressant in the non-immunised animal to maintain self-administration (R. L. Balster, personal communication). A saline substitution extinction of lever pressing for cocaine was interposed at this time and as before had no appreciable effect on heroin self-administration. The

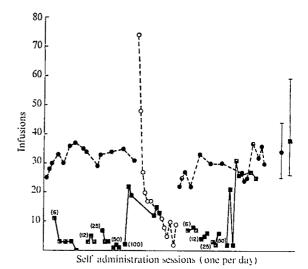


Fig. 2 Effect of immunisation on drug self-administration by a rhesus monkey, and effect of substitution of saline for cocaine after immunisation. Total infusions taken per 2-h session are shown. , Heroin infusions and heroin doses in µg per kg per infusion shown in parentheses; •, cocaine 100 µg per kg per infusion; O, saline infusions with cocaine-associated discriminative stimuli. Mean and range of drug infusions per 2-h session before immunisation are shown.

animal was then returned to the 6 µg kg<sup>-1</sup> heroin dose and the number of infusions taken per session again markedly decreased. In a second ascending series of heroin doses, self-administration was reinstated at 50 µg per kg per infusion with an average of 26 infusions taken per 2-h session (Fig. 2). The increased number of infusions taken by the monkey the second time drug self-administration was reinitiated compensated for the reduced dose at which the reinitiation occurred. As Fig. 3 shows, when heroin self-administration was maintained after immunisation the monkey received approximately 1-2 mg per kg per 2-h session, an amount of heroin about 10 times greater than that necessary to maintain self-administration before immunisation.

During these studies the concentration of antibody decreased. As Fig. 3 shows, serum obtained at least 18 h after the heroin sessions showed a decrease in capacity to bind 14C-morphine from 77,550 pmol ml<sup>-1</sup> undiluted serum\*to 7,900 pmol ml<sup>-1</sup>, approximately 10% of its former level. This decline in antibody available to bind incoming heroin may account for the reinitiation of heroin self-administration at the 50 µg per kg per

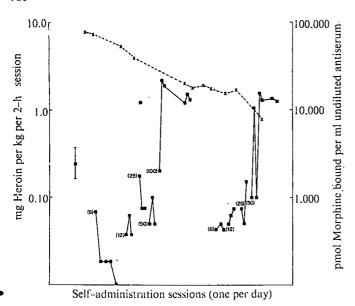


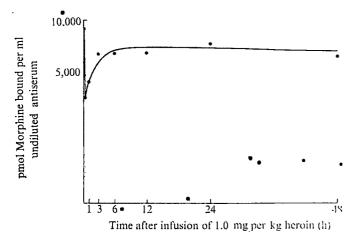
Fig. 3 Heroin intake and serum morphine-binding capacity after immunisation. , Heroin intake. Heroin doses in μg per kg per infusion are shown in parentheses.  $\times$ , Morphine binding capacity. Mean and range of heroin intake before immunisation are shown.

infusion dose in the second series of ascending heroin doses, although a learning effect cannot be ruled out. This decline in antibody concentration also shows that as in other immune reactions opiate antigen is non-immunogenic when bound by antibody in the presence of antibody excess13.

We next studied the time course of the heroin-antibody interaction by giving the monkey a single intravenous infusion of 1.0 mg kg<sup>-1</sup> over 10 min and obtaining frequent serum samples during the next 2 d. The results summarised in Fig. 4 show that the antibody concentration decreased sharply 6 min after heroin infusion. By 3 h it had risen again to approximately three-quarters of the starting concentration and there was little change during the next 48 h. The heterogeneity of antigen-binding we observed is consistent with an earlier finding of both high and very low affinity antibody in M-6-HS-BSA-immunised rabbits14.

Cerebrospinal fluid (CSF) was obtained by lumbar puncture to learn whether the antibody blockade of heroin extended into the CNS. The CSF bound 600 pmol morphine ml<sup>-1</sup> undiluted fluid. The capacity of the serum to bind morphine, determined at the same time, was 10,899 pmol ml<sup>-1</sup>, suggesting that most heroin binding takes place in the peripheral circulation.

The monkey was then allowed to self-administer only cocaine for 2 weeks, during which two booster immunisations were given. Four days after the second booster the monkey stopped



Serum morphine-binding capacity after intravenous infusion of 1.0 mg kg<sup>-1</sup> heroin. Fig. 4

self-administering cocaine. This marked the onset of a febrile illness which ended in the monkey's death 1.5 months later. Autopsy revealed widespread intranuclear inclusions in endothelial tissue supporting a diagnosis of disseminated viral infection. The kidneys showed hypercellular glomeruli with herpesvirus-like inclusions. Electron microscopy revealed intact basement membranes without areas of high electron density. These findings suggest that the monkey's death was not directly related to immunisation with M-6-HS-BSA.

Our results indicate that rhesus monkeys can be induced to produce antibody against opiates. The anti-opiate IgG is present in both serum and CSF. Blockade of CNS actions of heroin by this antibody can be demonstrated. Specifically, those actions of heroin that reinforce its self-administration in the non-dependent rhesus monkey can be blocked. This blockade has been shown to be dose dependent, and it can be overcome by high doses of drug.

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## β Alanine and cuticle maturation in Drosophila

THE sclerotisation and tanning of insect cuticles is generally thought to result from a crosslinking of the cuticular proteins by quinonoid derivatives of tyrosine<sup>1,2</sup>. Little consideration has been given to the fact that  $\beta$  alanine is a constitutent of the cuticles of many insects<sup>3,4</sup>, although it is required for normal tanning of the pupal case of several insects. Failure to utilise or produce the compound in Drosophila melanogaster results in an untanned pupal case<sup>5-7</sup>, whereas in Musca domestica, Bombyx mori and Drosophila virilis, failure to incorporate β alanine results in abnormally black cases. In this work, we suggest an explanation for these observations and provide evidence that  $\beta$  alanine also plays a role in the tanning of adult fruit-flies.

Microinjection experiments were carried out as described elsewhere". About 5 nmol of a sterile solution of β alanine (0.1 M) in insect Ringer<sup>8</sup> were injected into the abdomens of pharate adults 0-24 h before eclosion. The microinjection of β alanine into pharate adults of the mutant black produced a striking permanent phenocopy of the normal body colour. Genotypically black organisms injected with insect Ringer developed the typical mutant body colour.

Recently we reported that substantial amounts of dopamine accumulate prior to eclosion in D. melanogaster<sup>9</sup>. Oxidation of this dopamine to indole-5.6-quinone would be expected when O2 becomes available after eclosion, and melanisation would ensue if the indole-5,6-quinone molecules polymerised10. Because melanin deposition over a large part of the adult cuticle of D. melanogaster does not normally occur, we postulated that extensive polymerisation was prevented by  $\beta$  alanine. On this basis, the excessive pigmentation in the mutant black can be accounted for in terms of its failure to produce β alanine<sup>6</sup>. This contention is supported by our observation here that the injection of  $\beta$  alanine into black before eclosion prevents the mutant adult body colour from developing.

A result analogous to that obtained here for black adults has been reported for the black puparium (bp) mutant of Musca domestica. Feeding larvae of this mutant on food supplemented with ß alanine causes a normal (brown) puparium to form. We suggest that in those insects where the potential for abnormal melanisation at pupariation or eclosion exists (as demonstrated by the existence of mutants with excessively melanised cuticles), indole-5,6-quinone in conjunction with  $\beta$  alanine is required for normal tanning. Whether or not indole-5,6-quinone is involved in cuticle maturation at a particular stage in any insect would depend on the extent to which dopamine accumulates at this stage. In D. melanogaster the amount of dopamine present at pupariation is very low, which is consistent with the fact that no mutant of this genus having a dark pupal case is known.

This model readily provides a possible mechanism for the extensive melanisation which does occur in the stripes along the posterior margin of the tergites of wild type D. melanogaster. The obvious correlation between the location of the oenocytes and the pattern of melanin deposition in the abdominal cuticle11 (R.H., and A.C., unpublished) implicates these cells in melanin formation. Extensive indole quinone polymerisation would occur in the cuticle over the oenocytes if these cells did not secrete β alanine. Alternatively, a modification of the cuticular proteins secreted by the oenocytes so that they were unable to bind the indole quinones would also result in extensive melanisation, as experiments on ebony, suggest β alanine is unable to interact with unbound indole quinones to prevent their polymerisation.

Further study is needed on the role of  $\beta$  alanine in cuticle maturation, for we feel that the presently accepted description of tanning and sclerotisation in many insects is incomplete.

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#### Spermatozoa motility in human cervical mucus

THE study of spermatozoa penetration and migration into the cervical mucus is of prime importance in the understanding of human reproductive physiology. Cervical mucus is a heterogeneous secretion, the most important constituent of which is a hydrogel made of glycoproteic mucoids<sup>1</sup>. Changes in the arrangement of these glycoproteins seem responsible for the variation in the ability of spermatozoa to penetrate throughout the oestrous cycle. At ovulation or under oestrogenic stimulation, the macromolecules are grouped into parallel micelles which surround spaces containing a fluid of low viscosity<sup>2</sup>. The penetration of spermatozoa into the mucus is entirely dependent on their own motility.

Various tests have been described to study this penetration: the post coital test provides an estimate of the spermatozoa penetration in vivo but only allows a very incomplete appreciation of migration intensity and of movement quality. In vitro tests enable a dynamic study of spermatozoa penetration into the cervical mucus to be made and a simultaneous spermogram provides an estimate of the sperm quality.

When drops of semen and cervical mucus are placed in contact on a microscope slide, the spermatozoa form phalanges at the interface, enter the mucus and then move at random. If, however, the mucus is placed in capillary tubes, an orientated movement of the spermatozoa is observed. In these conditions the migration rate can be evaluated and the corresponding speed ranges from 1.6 to 50  $\mu m$  s  $^{-1}$  (ref. 3).

To obtain a complete, quantitative and objective evaluation of the migration of spermatozoa into cervical mucus, we have developed a method based on the Doppler effect. When an object moving at speed v receives monochromatic light having a frequency vo, it scatters the light which undergoes a frequency change  $v = v_0(2v/c\cos\alpha\sin(\theta/2))$  where  $\theta$  is the angle between the incident beam and the direction in which the scattered light is observed,  $\alpha$  is the angle between v and the exterior bisector of  $\theta$ , and c is the speed of light. For a fixed value of  $\theta$  and when  $\nu$  and  $\alpha$  are single,  $\nu = k\nu \cos \alpha$  has only one value (k being constant). When several objects move at different speeds, but in the same direction (a fixed), the frequency change gives rise to a frequency spectrum S(v). directly representative of N(v); N is the number of objects moving at the speed  $v = v/(k \cos \alpha)$ . When, moreover,  $\alpha$  is unrestricted, the frequency change gives a more complex spectrum; in this case N(v) is represented by the function vdS(v)/dv.

The frequency shift due to the movement of spermatozoa is very small and is detected by a heterodyne technique<sup>4</sup>. The incident light is a He-Ne laser beam; a phototube receives the scattered light from the moving object and a small part of the incident light simultaneously. Beats between these two lights give rise to fluctuations in the photocurrent and the analysis of them (by a Fourier analyser) gives S(v) directly. The cervical mucus is taken with a syringe attached to a pipette made of optical quality glass which acts as a scattering cell. This pipette is 150 mm long of cross section 8 x 3 mm and contains along its axis a central tube 1 mm square. The fine end of the pipette is introduced into the external cervical os and the mucus is aspirated gently. The pipette is then placed for 30 min in a test tube containing 0.3 ml of the sperm to be studied.

To study migration in cervical mucus, the pipette is placed perpendicular to the incident beam which travels through it parallel to the shortest side (e = 3 mm), the scattered light is observed at an angle  $\theta = 8^{\circ}$  so  $\alpha = 4^{\circ}$  and  $\cos \alpha \simeq 1$ . To evaluate motility of the spermatozoa in the semen itself, a flat

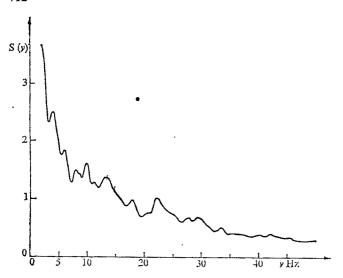


Fig. 1 Frequency spectrum S(v) (arbitrary units) given by the sperm S<sub>1</sub> (60×10<sup>6</sup> spermatozoa ml<sup>-1</sup>; normal motility, 60%; reduced motility: 15%).

cell is used, 15 mm in diameter and having an optical path of 0.1 mm. The plane of the pipette is placed perpendicular to the incident beam, the power of which is less than  $0.1 \times 10^{-3}$  W. The temperature of the cell (pipette or plan cell) is 37° C.

Analysis of the light scattered by the spermatozoa moving in the seminal plasma gives a spectrum S(v) (Fig. 1) which is wide and monotonically decreasing as v increases. The distribution  $N(v_s)$  of instantaneous speed  $v_s$  in the sperm, calculated from this spectrum, is shown in Fig. 2. This wide distribution demonstrates the complexity of spermatozoa movements such as are observed under the microscope when a drop of semen is placed on a lamella.

In constrast, the spectrum representing the movement of spermatozoa in cervical mucus is a remarkably well defined line at a frequency value shifted with respect to  $\nu=0$  (Fig. 3A). This kind of spectrum is unambiguously significant of an

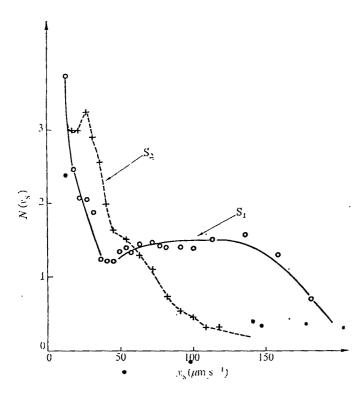


Fig. 2. Velocity distribution  $N(\nu_s)$  (arbitary units) against velocity  $\nu_s$  for sperm  $S_1$  and  $S_2$  (for the sperm  $S_2:94.4\times10^6$  spermatozoa ml<sup>-1</sup>; normal motility: 65%; reduced motility: 5%).

orientated unidirectional movement of the spermatozoa. First of all, this result strongly confirms the spermatozoa migration in cervical mucus observed under the microscope; furthermore, the spectrum gives directly the distribution of the spermatozoa speeds  $V_{\rm M}$  in the mucus, the speed being related to frequency by  ${\rm v}=k\nu$ . By its characteristics (high and well defined speed,  $V_{\rm M}=50\pm10~{\rm \mu m~s^{-1}}$ ), the spectrum in Fig. 3A gives evidence of the good 'mutual' quality of the cervical mucus (orientated structure) and of sperm  ${\rm S}_1$  (high instantaneous speed of the spermatozoa).

The important part played by the sperm quality in the migration through mucus has been pointed out by a complementary experiment. The same mucus as in the previously related experiment has been penetrated by the spermatozoa of another sperm ( $S_2$ ) the means speeds  $\nu_s$  of which, in the seminal plasma, are clearly lower than in the case of the sperm  $S_1$  (Fig. 2). In cervical mucus, movement of the spermatozoa of sperm  $S_2$  gives a spectrum (Fig. 3B) which is very different from that obtained with  $S_1$  (Fig. 3A). The frequency peak is less well defined and remains in very low frequencies: its maximum corresponds to low speed  $V_M$  between 5 to 10  $\mu$ m s<sup>-1</sup>. The relationship between the instantaneous speed  $\nu_s$  in the seminal plasma and

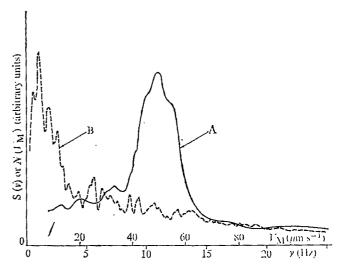


Fig. 3 Frequency spectrum (and velocity distribution) relative to the motion of the spermatozoa in cervical mucus. The measurement has been made 0.5 h after the beginning of the penetration and at 4 cm from the fine end of the pipette. A, sperm  $S_1$ ; B, sperm  $S_2$ .

the migration speed in the mucus is not maintained. In the case of the  $S_1$  spermatozoa,  $V_s$  is 100  $\mu$ m s<sup>-1</sup>, compared with  $V_M$  in cervical mucus, which is 50  $\mu$ m s<sup>-1</sup>. For the  $S_2$  spermatozoa,  $v_S$  is 30  $\mu$ m s<sup>-1</sup>, when  $v_M$  in mucus is approximately estimated as 8  $\mu$ m s<sup>-1</sup>. This indicates the functional importance of the global and objective measurement of spermatozoa velocities in cervical mucus, which points out the importance of the spermatozoa motile quality.

We believe that the scattering light technique related to the Doppler effect gives an excellent tool for the measurement of spermatozoa speeds. Objective velocimetry is of fundamental importance in the study of living motile microorganisms. If the results obtained in the particular cases of sperm S<sub>1</sub> and S<sub>2</sub> are compared by this method and by microscopic observation, the latter test indicates an apparently better quality for sperm S<sub>2</sub> (see Fig. legends) and this estimation, though performed by specialists, is shown to be wrong according to the objective results given by our method. In the case of migration of spermatozoa in cervical mucus, our method provides a total appreciation of the migration with a direct, simple and quick measurement of the speeds. Moreoever, the results confirm that the migration is orientated at the time of

ovulation. Our study corresponds to the first observation by Doppler effect of a type of orientation of living microorganism movement, which is not due to a taxis<sup>5-7</sup> but to mechanical guidance.

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## Separation of parathyroid hormone and calcitonin-sensitive cells from non-responsive bone cells

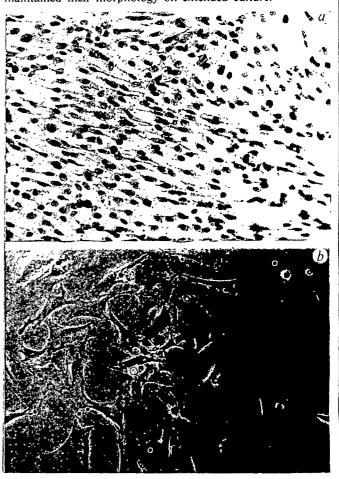
BONE growth and remodelling depend on resorption and deposition at the local level. The balance between these two processes seems to be modulated by the peptide hormones PTH and calcitonin. Parathyroid hormone (PTH) has been shown to induce bone resorption both in vivo1 and in vitro2, whereas calcitonin (CT) inhibits this process<sup>3</sup>. Recent studies suggest that both agents act on cellular mechanisms through a second messenger. Thus, it has been demonstrated that exposure of mouse calvaria to PTH or CT can lead to an increase in cyclic AMP content<sup>4,5</sup>. In addition, dibutyryl cyclic AMP can by itself induce bone resorption<sup>2</sup>.

An understanding of the molecular mechanisms which control synthesis and degradation of bone matrix is lacking. This is a result partly of the complexity of bone tissue in which there exists within the large volume of extracellular matrix a variety of cellular types including osteocytes, osteoblasts, osteoclasts, fibrocytes and marrow elements<sup>6</sup>. Morphological changes suggest that bone cells can respond to both PTH7 and CT8. But, it is not known which of these cells is the primary target for the action of these hormones. Moreover, the fundamental question of whether PTH and CT act on the same or different cell types, is open. Recent efforts to isolate bone cells have resulted in mixtures of cellular types<sup>9,10</sup> which have been used immediately after isolation. Attempts to maintain these cells in culture have resulted in rapid overgrowth by fibroblasts<sup>9</sup>.

A clear elucidation of the molecular events triggered by PTH and CT should be aided by the isolation and identification of the various cellular types. Here we report the isolation of a selected population of bone cells from mouse calvaria which exhibit and retain in culture for several days distinct morphological and biochemical characteristics.

Freshly excised calvaria (usually 25 in number and weighing about 100 mg) were minced and extracted by stirring for 20 min in an isotonic, calcium-free salt solution containing 0.1% collagenase, 0.05% trypsin and 0.005 M EDTA (Table 1). The cells released into the extract were collected by centrifugation at 10,000 r.p.m. for 5 min (population 1). This extraction was repeated a second and a third time to yield cell populations 2 and 3. The yields of cells were  $1 \times 10^6$ ,  $2 \times 10^6$  and  $2 \times 10^6$  in populations 1, 2 and 3 respectively. DNA measurements established that 20% of the total calvarial DNA had been extracted. Samples of calvaria were examined histologically with haematoxylin and eosin and Von Kossa stains after each step of the extraction procedure. There was a high degree of variability in appearance from sample to sample and within the same sample of tissue. The general impression was that the extraction progressively removed cells from the periosteal surface of the calvaria inward such that there was an increasing number of empty lacunae. After the third extraction osteocytes buried in the calcified matrix still remained, as did most of the cartilage and chondrocytes. These remaining cells comprised the bulk of those present in the control samples in agreement with the analyses for DNA.

Cells of each population were placed in monolayer culture at a density of 2×106 cells per culture flask. In addition, the calvarial tissue remaining after the third extraction was also placed in culture. Populations 1, 2 and 3 all exhibited a similar morphology immediately upon isolation. This similarity persisted for up to 48 h in culture and appeared as a mixture of small rounded and stellate cells. After 5-6 d, differences in morphology and growth pattern became evident in the three populations. Population 1 rapidly divided and by 7-10 d spindle shaped cells resembling fibroblasts predominated in the culture (Fig. 1a). The morphology of population 3, in contrast, was transformed into relatively large cells with a highly convoluted dendritic border (Fig. 1b). Cellular division was slow and this morphology persisted for at least 2 weeks. Population 2 at first exhibited morphological characteristics of both populations 1 and 3, but by the 14th day the spindle shaped cells had overgrown the culture. In the case of the cultured calvarial remnants, cells migrated out from the tissue (population 4). Morphologically, these cells resembled closely those of population 3 in their stellate appearance but were somewhat larger. These cells also maintained their morphology on extended culture.



Photographs of bone cells population 1 (a) (fixed and Fig. 1 stained in haematoxylin and eosin) and population 3 (b) 7 days after extraction from mouse calvaria ( $\times$  200).

Table 1 Response of bone cell populations to PTH and CT

	Table 1 Response of Jone cent populations to 1111 and C1					
-			Cyclic AMP (pmo)			
	Sample	Control	PTH	SCT	PTH 0.44 U ml <sup>-1</sup>	
	•		0.44 U ml <sup>-1</sup>	0.25 U ml <sup>-1</sup>	SCT 0.25 U ml <sup>-1</sup>	
	Unextracted calvaria	$25 \pm 1$	227 ± 4*	50 ± 5*	248 :: 4*	
	Extracted calvarial remains	28 + 4	48 6*	32 4	48 3*	
	Population 1	20 + 3	21 - 3	15 - 2	19 - 3	
	Population 2	$\frac{1}{21} \stackrel{\perp}{=} \frac{1}{2}$	125 - 5*	38 : 4*	141 - 6	
	Population 3	24 - 4	236 7*	65 - 7*	260 7	
	Population 4	$\frac{27}{27} \pm \frac{7}{3}$	56 ÷ 3*	$32 \pm 5$	57 - 6*	
	r opulation 4	21 = 3	JU == J.	34 ::: 3	37 <u>=</u> 0	

Calviaria from 2-3-d-old mice were minced and suspended with constant stirring in 5 ml of a sterile salt solution consisting of 136.0 mM NaCl, 2.6 mM KCl, and 0.36 mM NaH<sub>2</sub>PO<sub>4</sub>, containing 0.1% collagenase (180 U mg<sup>-1</sup>, Worthington Crude), 0.05% trypsin (BBL lyophilised powder 2.5%) and 5 mM EDTA, pH 7.0. The cells released into solution after 20 min were collected by centrifugation at 10,000 r.p.m. for 5 min at 20° C (population 1). Fresh enzyme solution was added to the calvarial remains and the procedure was repeated twice to give to cell populations 2 and 3. These were placed in monolayer culture in Minimal Eagles Medium nutrient mixture (Gibco) supplemented with 10% foetal calf serum (Gibco), 100 U ml<sup>-1</sup> penicillin, 100 mg ml<sup>-1</sup> streptomycin and 2.2 mg ml<sup>-1</sup> Na bicarbonate. The calvarial remains were either assayed immediately for hormonal response or were placed in culture to yield population 4. After 7 d in culture, 5 × 10<sup>5</sup> cells from each cell population were inoculated into culture dishes. The standard growth medium was decanted 6 h later and fresh medium containing 5 mM theophylline and PTH (0.44 U ml<sup>-1</sup>) or SCT (0.25 U ml<sup>-1</sup>) was added to the monolayers. The reaction was stopped 5 min later with two rapid rinses in ice cold Tyrode's salt solution. 0.8 ml of ice cold ethanol-0.2 N HCl was added, and the cells were collected by scraping the culture dishes. After 24 h at -10° C the extracts were centrifuged. The supernatants were removed, dried in vacuo, and cyclic AMP measured by the method of Gilman<sup>11</sup>. Protein kinase and protein kinase inhibitor were obtained from Sigma. All determinations were performed in duplicate at two different concentrations of cyclic AMP. Known amounts of cyclic AMP were added to aliquots of each extract as internal standards, and recovery was more than 90%. The pellets were heated in 10% perchloric acid and DNA was measured by Burton's modification of the diphenyl reaction<sup>12</sup>. Each number in the table represents the average ± s.e.m. of 6-8 separate studies.

\*Dif

The four cell populations and fresh unextracted and extracted calvaria were tested for their ability to produce cyclic AMP in response to the bone-active hormones PTH and CT (Table 1). As previously established<sup>4,5</sup>, both hormones rapidly elicited severalfold increases in cyclic AMP content of intact calvaria. The response to PTH was about fourfold greater than to CT at maximally effective concentrations. The effects of the two hormones were additive. After the removal of populations 1-3, however, the calvaria responded only minimally to PTH and not at all to CT suggesting that essentially all of the hormone-sensitive cell populations had been removed by the extraction procedure. Among the cultured isolated bone cells, population 3 exhibited the greatest responsiveness to both PTH and CT. Populations 2 and 4 were substantially less responsive, and population 1 was not responsive. The ratio of the increases in cyclic AMP produced by each hormone was about the same in the responding cell populations as it was in the fresh calvaria. Moreover, the effects of the two hormones were additive.

The action of a few other hormones on the separated cell populations was examined (Table 2). ACTH and MSH had no effect on cyclic AMP levels on the three populations, whereas PTH, CT and adrenaline (which has been reported to affect the level of cyclic AMP in bones<sup>10</sup>) all increased the cellular content of this nucleotide in population 3 and to a smaller extent in population 2. As before, population 1 was unresponsive.

On extended culture, the maximum responsiveness of population 3 to both PTH and CT individually and in combination

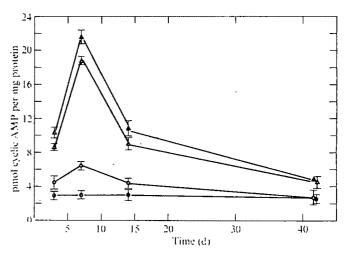


Fig. 2 Loss of response to PTH snd CT upon prolonged culture of population 3. At various times after establishment in culture, 2×10<sup>3</sup> bone cells from population 3 were grown under standard conditions with no additions, (♠); PTH 0.44 U ml<sup>-1</sup>, (△); SCT 0.5 U ml<sup>-1</sup>, (○) or PTH (0.44 U ml<sup>-1</sup>) and SCT (0.5 U ml<sup>-1</sup>), (♠) in media supplemented with 5 mM theophylline. After 3 min the reaction was stopped by rapid rinsing of the cells with ice cold Tyrode's salt solution. The cells were then processed for cyclic AMP measurements as described in Table 1. Protein was measured by the method of Lowry et al.<sup>13</sup>. The bars for each data point represent: s.e.m.

Table 2 Specificity of hormonal response

Agent			Cycli		) per 100 µg DNA opulation	4
None PTH (0.44 U ml <sup>-1</sup> ) SCT (0.5 U ml <sup>-1</sup> ) L-adrenaline (5 × 10 <sup>-8</sup> M) ACTH (0.35 U ml <sup>-1</sup> ) MSH (10 <sup>-8</sup> M)	٠.	$   \begin{array}{c}     48 \pm 7 \\     40 \pm 10 \\     34 \pm 2 \\     39 \pm 1 \\     40 \pm 2 \\     35 \pm 3   \end{array} $	•	28 :: 4 49 ± 10* 37 :: 3† 31 ± 2 20 ± 5 25 ± 4	3 22 ± 2 161 ± 20* 65 ± 7* 71 ± 13* 25 ± 3 30 ± 2	22 ± 5 41 ± 10* 28 ± 3 42 ± 5* 18 ± 2 21 ± 3

Specificity of hormonal response. Seven days after establishment in culture, bone cell populations 1, 2, 3 and 4 were subcultured in 5 mM theophylline and PTH (0.44 U ml<sup>-1</sup>), ACTH (0.35 U ml<sup>-1</sup>), MSH ( $10^{-8}$  M), SCT (0.5 U ml<sup>-1</sup>) or L-adrenaline (8 ×  $10^{-6}$  M) was added for 10 min. The control nedium contained only theophylline. The reaction was stopped by the addition of ice cold Tyrode's salt solution. Cells were rinsed and processed for cyclic AMP measurement as described in Table 1. MSH was a gift from Dr J. Pawalek, and ACTH and L-adrenaline were purchased from Sigma.

\* Differs significantly from control P < 0.01

<sup>†</sup> Differs significantly from control P < 0.01

reached a peak at 7 d and then declined (Fig. 2). After 40 d the response to PTH was slight and that to CT was not detectable. Peak responsiveness occurred at the time when the distinctive morphology illustrated here (Fig. 1b) was well established.

These results indicate that a selected population of bone cells may be obtained by sequential extraction which seem to be specific targets for PTH and CT. To our knowledge this is the first time that such a separation has been demonstrated.

The present data which show an additive effect of PTH and CT on cyclic AMP levels suggest that non-competing receptor sites for both hormones are present in population 3. The coextraction of PTH and CT-responsive cells (Table 1), the simultaneous peaking of the responses at one week in culture (Fig. 2), and the simultaneous loss of this responsiveness on extended culture (Fig. 2) suggest that the receptors to PTH and CT reside either in the same cell, or in different cells which exhibit a similarity in their extractability from bone and viability in culture. Efforts are under way to further fractionate the cells in population 3 (on the assumption that this is a heterogeneous population), and to investigate some of the molecular events initiated by these hormones.

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## Presence of a gonadotrophin on the surface of preimplanted mouse embryos

In humans, a gonadotrophin (hCG) is localised on the cell membrane of the syncytiotrophoblast facing the maternal bloodstream<sup>1</sup>. The syncytiotrophoblast is derived from the trophoblast cells of the mammalian blastocyst and is thought to participate in isolating the foetus from attack by the maternal immunological system<sup>2</sup>. Martin, et al. suggest that the hCG on human syncytial plasma membranes may, in part, be responsible for the lack of maternal immunological rejection of the foetus; in addition to its role in steroidogenesis, therefore, hCG may also have an immunological function during pregnancy. Because hCG is generally used to induce super-ovulation in mice and must, therefore, cross-react with mouse gonadotrophin, and since the mouse is extensively used in studies on mammalian development, I investigated the possible presence of gonadotrophin(s) in mouse embryos before implantation and formation of the syncytiotrophoblast. Using an antiserum to hCG and indirect immunofluorescence (IIF) I have detected a luteinising hormone (LH)- or hCG-like substance ('embryonic gonadotrophin', EG) on the cell surfaces, of live mouse preimplanted embryos.

Random-bred SWR female mice 6-8 weeks old were superovulated and mated to provide unfertilised eggs and various in vivo preimplantation stages for the IIF assays3. Superovulated BALB/c females mated with C57BL males, and naturally-ovulated ICR female mice mated with C3H males, provided additional in vivo morulae and blastocysts. Two-cell embryos from super-ovulated SWR mice were collected and cultured to blastocysts, using standard techniques4.5, to provide in vitro preimplantation stages. Blastocysts which had been collected from SWR mice and allowed to attach to, and grow on, glass slides were also examined. The IIF assay consisted of a 30-min incubation in rabbit anti-hCG (Miles Laboratories) diluted 1:1 with phosphate buffered saline (PBS), two 5-min rinses in culture medium, a 10-min incubation in goat antirabbit IgG conjugated with fluorescein (Antibodies, Inc.) diluted 1:4 with PBS, and two 5-min rinses in culture media to remove unbound conjugate. Slides of fixed mouse placenta (16-18 d), human second-trimester placenta, and mouse ovary were assayed for IIF-detectable gonadotrophin using anti-hCG and anti-rabbit IgG fluorescein conjugate as previously described. IIF controls were incubated in either normal rabbit serum followed by flourescein, or in the fluorescein conjugate only. Most of the stages were assayed in triplicate. Embryos were placed in a small amount of culture medium on glass slides, and the slides of tissue sections were viewed with a Zeiss fluorescence microscope and photographed using Agfa GAF 500 colour slide film (exposures of 1 min).

Before the eight-cell stage, little gonadotrophin was detected on the cell surface on any of the stages (Fig. 1). Then, only a few isolated blastomeres of an occasional embryo would show light fluorescent outlines. Cell surfaces of morulae, however, were strongly positive for gonadotrophin and remained positive as blastocoel formation ensued. With blastocyst expansion. however, fluorescence diminished to pre-morulae levels and was not detectable on blastocysts which had attached to, and grown on, glass. Results for embryos cultured from the two-cell stage were similar except that the marked increase in positive fluorescence observed with the morulae was depressed slightly and persisted throughout blastocyst hatching. No consistent differences in the pattern of positive fluorescence was observed between embryos taken from naturally or super-ovulated mice,

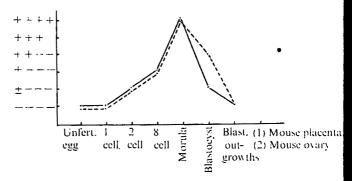


Fig. 1 Cell-surface gonadotrophin detected by IIF on mouse preimplantation embryos. The impressions of three observers who scored the embryos for positive fluorescence were averaged to produce the differences in fluorescence assigned to the preimplantation stages. The symbols (--) and (++represent the total lack, and the greatest amount, respectively, of IIF-detected cell-surface gonadotrophin. Gradual replacement of plus signs with minus signs indicate diminishing levels of fluorescence. In vivo embryos, -; cultured embryos,

or from mice of different strains. No anti-hCG binding was observed on mouse 16-18 d gestation placenta, or mouse ovary. Positive fluorescence was observed on sections of human second-trimester placenta, and was confined to the syncytiotrophoblast of the chorionic villi.

Existing opinion holds that production of EG (gonadotrophin secreted by the placenta or its derivatives) is confined to the syncytiotrophoblast. The rabbit blastocyst has, however, been shown to contain a gonadotrophin which competes with hCG in binding assays<sup>7</sup>, and to secrete an LH-like substance by radioimmunoassay<sup>8</sup>. These observations, together with the high maternal plasma levels of hCG observed at about the time of implantation<sup>9</sup>, indicate that EG may be produced before implantation and formation of a syncytium. The observation that anti-hCG binds to morulae cultured from two-cell embryos suggests that, in addition to having a trophic hormone(s) on their cell surfaces (irrespective of the origin of the hormone), mouse preimplantation embryos may synthesise at least some of the gonadotrophin found on their surfaces.

Besides initiating steroidgenesis, cell-bound gonadotrophins may play a role in mediating protection of the conceptus against maternal rejection during implantation. Such a role has been suggested for hCG, which reversibly inhibits PHAstimulation of human lymphocytes in vitro 10 and is found in high concentrations in plasma throughout pregnancy. It is not known whether EG has a similar role in rodents nor whether chorionic gonadotrophin is present in rodents, including the mouse (A. Contopoulos, personal communication). No antihCG binding was detected on blastocyst outgrowths, nor on mouse placenta, whereas human placenta similarly fixed and assayed was positive for anti-hCG binding. Perhaps a gonadotrophin is present in mouse placenta, but in a form not recognised by anti-hCG.

It is now widely thought that polypeptide hormones achieve their effects by first interacting with the cell membrane or with membrane-bound enzymes11. hCG binds to the cell membranes of luteinised rat ovaries and presumably such binding precedes the onset of ovarian steroidogenesis<sup>12</sup>. Initiation of steroidgenesis found in the preimplantation embryos of the rat13 and rabbit14 may also require the presence of cell surface-bound gonadotrophin. In addition, it has been suggested that steroidgenesis in the preimplanted rat embryo may be necessary for morulae to blastocyst transformation<sup>13</sup>. Similar processes may also occur in the mouse.

In conclusion, mouse preimplantation embryos display a gonadotrophin(s) on their cell surfaces, and IIF-detectable levels of this gonadotrophin(s) is maximal on morulae. In addition, I suggest that the preimplanted mouse embryo may be capable of synthesising at least some of the gonadotrophin found on their surfaces.

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### 1,25-dihydroxycholecalciferol-like activity of Solanum malacoxylon extract on calcium transport

CATTLE grazing the South American egg plant, Solanum malacoxylon, develop hypercalcaemia and hyperphosphataemia resulting in severe calcinosis1. The active principle in the plant stimulates intestinal calcium transport2-4 and bone mineral resorption<sup>2,4</sup> in laboratory animals. It resembles vitamin D except that it is water soluble1.5 and exerts its initial action and declines in activity more rapidly2. Recent work6 suggests that the factor closely resembles 1,25-dihydroxycholecalciferol (1,25-(OH)<sub>2</sub>-D<sub>3</sub>), a metabolite of vitamin D<sub>3</sub> formed in the kidney<sup>7</sup>, in that it overcomes the inhibition by dietary strontium<sup>8</sup> of vitamin D<sub>3</sub>-induction of the intestinal calcium-binding protein (CaBP) and, concomitantly, calcium absorption9.

Here we demonstrate that the S. malacoxylon factor stimulates the calcium absorptive mechanism of the organ-cultured chick duodenum, indicating that the activity of the factor does not depend on preliminary metabolism by some other tissue, that, indeed, it resembles 1,25-(OH)2-D3 in terms of its biopotency, and that its mechanism of action, like that of both vitamin D<sub>3</sub> itself and 1,25-(OH)<sub>2</sub>-D<sub>3</sub>, is dependent on intestinal protein

Technical details of the organ-culture system, assay of CaBP and measurement of calcium uptake and transport in the cultured duodenum have been published elsewhere 10-12.

After preliminary experiments proved that S. malacoxylon extract (Fig. 1 legend) was effective in eliciting vitamin D-like responses in organ-cultured duodena, a time study was conducted (Fig. 1). Within 12 h of culture, the extract induced CaBP synthesis and stimulated 45Ca uptake. There was excellent correlation between CaBP concentration in the tissue and the ability of the tissue to accumulate radiocalcium. This experiment confirmed the vitamin D-like activity of the Solanum factor as only vitamin D<sub>3</sub>, and related sterols<sup>10-13</sup>, are effective in inducing CaBP in this system. The lag time observed was more reminiscent of vitamin D<sub>3</sub> itself (which is effective without transformation in this system<sup>11</sup>) than 1,25-(OH)<sub>2</sub>-D<sub>3</sub> (refs 10 and 13). There is, however, ample evidence (for example, its water solubility<sup>1,5</sup>) that the active principle in S. malacoxylon is not a simple sterol (Peterlik and R.H.W., unpublished). The possibility exists, therefore, that some preliminary transformation of the principle must take place before its characteristic activity ensues but our results indicate that such change can occur in the intestine itself.

Other evidence supports the contention that the Solanum factor is more like 1,25-(OH)2-D3 than vitamin D3. The doseresponse relationship for Solanum extract was compared with that of authentic 1,25-(OH)2-D3, by far, the most potent sterol for CaBP induction in this system<sup>10,11</sup>. In terms of CaBPinducing potency, 0.01 and 0.1% concentrations of Solanum extract were equipotent to 65 and 650 pM 1,25-(OH)<sub>2</sub>-D<sub>3</sub>, respectively (Table 1). This means that there were 650 pmol of 1,25-(OH)2-D3-like activity in each ml of Solanum extract, or 365 pmol of 1,25-(OH)<sub>2</sub>-D<sub>3</sub>-like activity per g of powdered leaf.

Another experiment confirmed the 1,25-(OH)<sub>2</sub>-D<sub>3</sub>-like

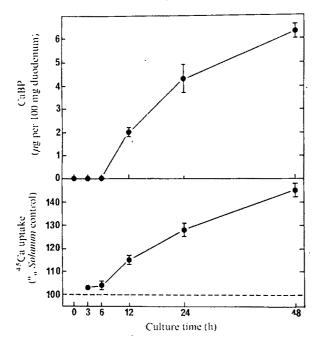


Fig. 1 Time study of the effect of S. malacoxylon extract (0.1% of the culture medium) on CaBP synthesis and stimulation 6 of the culture medium) on CaBP synthesis and stimulation of \*\*Ca uptake. Values are the mean ± s.e. The S. malacoxylon extract used in these experiments was prepared by shaking the dried, ground leaf three times with three volumes of methanol: chloroform (2:1) for 2 h at room temperature discarding the solvent each time. After residual solvent removal by aeration, the residue was shaken with nine volumes of water for 16 h The aqueous extract was filtered through cheese-cloth and centrifuged (24,000g for 20 min). The supernatant was lyophilised, shaken for 16 h in 75% ethanol in water and centrifuged. The ethanolic extract was concentrated sixfold and stored at -20° C. This procedure effected a 42-fold concentration of the active principle over the powdered leaf. Activity of the various fractions was assayed by determining CaBP production when administered to rachitic chicks 4 weeks old18. Aliquots of this final concentrated extract were added to the culture medium as indicated. Duodena were cultured slit-open on grids<sup>10,11,13</sup> mucosal-side-up for the times indicated, in the presence of either the extract or extracting solvent alone (75%) EtOH-control group) in the culture medium. CaBP assay and <sup>45</sup>Ca uptake were performed as previously described<sup>10,13</sup>. There was no CaBP present in duodena cultured in the presence of extracting solvent alone.

activity of the Solanum factor in what has proven to be a new test system, that is, the embryonic chick duodenum in ovo. Some time ago, it was found that embryonic chick duodenum neither contains CaBP until the day of hatching (day 21) nor synthesises CaBP in response to the injection of vitamin D<sub>3</sub> directly into the egg14. Recent work has confirmed this original observation while demonstrating that metabolism of vitamin D<sub>3</sub> to its more active metabolites does occur in the embryo15. We here report that injection of 1,25-(OH)<sub>2</sub>-D<sub>3</sub> (300 pmol per egg) directly into the egg (at 18 d incubation) did induce precocious CaBP synthesis when measured 36 h after injection  $(8.3 \pm 0.9 \mu g \text{ CaBP per } 100 \text{ mg duodenum})$ . Interestingly, when Solanum extract was injected into each egg (an amount estimated to contain 325 pmol 1,25-(OH)2-D3-like activity; see Table 1), CaBP was also induced and to an almost theoretically predictable extent (8.9  $\pm$  1.7  $\mu g$  CaBP per 100  $\,$  mg duodenum). By contrast, 240,000 pmol vitamin D<sub>3</sub> (800 times as much 1,25-(OH)2-D3 on a molar basis) was completely without effect and 26,000 pmol 25-OH-D<sub>3</sub> was only marginally effective (3.2  $\pm$  0.6  $\mu g$  CaBP per 100 mg duodenum). The . embryonic chick duodenum is refractory to endogenous levels of vitamin D<sub>3</sub> and its metabolites, although possessing the inherent ability to respond to sufficient levels of these sterols both in organ culture<sup>10-13</sup> and, to the active metabolites, in ovo. This strongly suggests that stimulatory levels of the appropriate sterol are not reached in the duodenum in ovo. The reason for this is completely unknown.

The action of either vitamin D<sub>3</sub> (refs 12 and 13) or 1,25-(OH)<sub>2</sub>-D<sub>3</sub> (ref. 16) in the organ cultured duodenum has been shown to be an actinomycin D-sensitive process, that is, sterol action involves DNA transcription and subsequent protein synthesis. The Solanum factor seems to act by a similar mechanism as actinomycin D completely abolished the response to Solanum extract (Table 2). It is also interesting to note that not only 45Ca uptake by the duodenum, but also mucosal-toserosal transport were stimulated by Solanum and that both <sup>45</sup>Ca movements were inhibited by actinomycin D.

The foregoing experiments confirm and amplify previous work<sup>6</sup> indicating the 1,25-(OH)<sub>2</sub>-D<sub>3</sub>-like activity of the Solanum factor. There is no doubt that this factor functions by an actinomycin D-sensitive mechanism, similar to that of the D-vitamins, in inducing CaBP and stimulating calcium transport. There also can be little doubt that, with respect to its biopotency in culture and in ovo, the factor in S. malacoxylon extract resembles 1,25-(OH)<sub>2</sub>-D<sub>3</sub>. The recent report that aqueous extracts of Solanum failed to mobilise bone calcium in vitamin D-deficient rats fed a low calcium diet, while 1,25-(OH)<sub>2</sub>D<sub>3</sub> was effective<sup>17</sup>, is hard to reconcile with the present findings, those with strontium-fed chicks6, and those of earlier workers who did, in fact, show an action on bone mineral resorption<sup>2,4</sup>.

lative potency of S. malacoxylon 1,25-(OH)<sub>2</sub>-D<sub>3</sub> in the induction of CaBP extract and Relative Table 1

Concentration is	n medium	
S. malacoxylon	$1,25-(OH)_2-D_3$	CaBP per 100 mg
Extract (%)	(pM)	duodenum (μg)
0 (EtOH o	nly) 0	0
0.01		$3.5 \pm 0.6$
0.1		$7.8 \pm 0.7$
- was walking	65	3.4 :- 0.5
Arrange and	650	$7.4 \pm 0.5$

Values are mean ± s.e. Duodena were cultured slit-open on grids10. 11.13 mucosal-side-up for 48 h in the presence of the extract (prepared as given in the legend to Fig. 1) or the authentic sterol in the culture medium. Additions were made in EtOH such that the final EtOH concentration was 0.1%. CaBP assay was performed as previously described 10-13.

Finally, the active principle from Solanum malacoxylon, or, perhaps, from some abundant native plant, holds promise as an source of 1,25-(OH)2-D3-like activity. This may prove important given the difficulty and expense of producing synthetic 1,25-(OH)2-D3 and the widespread desirability of utilising 1,25-(OH)2-D3 for studies of animal and human disorders of calcium metabolism which may be refractory to vitamin D. itself. Isolation and purification of the active principle is of great importance and is proceeding in several laboratories. In our own laboratory, the organ culture system—and the in ova

Table 2 Inhibition of the action of S. malacoxylon extract or CaBP synthesis and radiocalcium uptake and transport in organ cultured duodenum by actinomycin D

			<sup>45</sup> Ca movement (% of zero	
Concentration in medium			extract control)	
		CaBP per		Mucosal→
S. malacoxylon	Actinomycin D	100 mg	Tissue	serosal
extract (0.1%)	(1.6 µM) đượ	odenum (µg)	uptake	transport
	_	0	100	100
+		$2.9 \pm 0.3$	145±5*	$176 \pm 12*$
	+	0	100	100
+	+	0	$97 \pm 4$	$107 \pm 12$

values are the mean±s.e. Duodena were cultured everted operids<sup>10,13</sup> for 48 h in the presence of *Solanum* extract. (Fig. 1 legend and actinomycin D in the culture medium. CaBP assay and measurement of <sup>45</sup>Ca movement were performed as previously described<sup>10,13</sup>.

\*Significantly greater than zero extract control at 1% leve Students' t test.

preparation—has proved to be of significant value in quantifying the factor during the various stages of its isolation.

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#### Malaria and the permeability of the host erythrocyte

WHEN an animal is infected with malaria some of the properties of the membrane of its erythrocytes are changed1. The fragility is increased2-6 and the transport of sodium, potassium and amino acids across the membrane is altered<sup>6-10</sup>. In most cases these alterations have been shown to be present in all erythrocytes from an infected animal and not just those containing parasites, which suggests a general pathological effect rather than a direct action of the parasite on its host cell.

We have found that erythrocytes from infected animals also show an increased permeability to L-glucose, a substance which does not readily penetrate either the membrane of erythrocytes from normal animals or that of immature red cells. This increased permeability could be related to the other membrane changes described above, and if so should be shown by both parasitised and non-parasitised erythrocytes from an infected animal. The complete separation of these two types of cell would be difficult, but an approximate separation can be made by centrifugation<sup>5,6,8</sup>, since those cells which contain parasites sediment more slowly than those which do not. We have therefore used this technique to determine whether all cells or only parasitised cells show an increased permeability to L-

Blood from 10-15 mice infected with Plasmodium berghei (a malarial parasite of rodents) was pooled, the white cells removed and the erythrocytes collected by centrifugation after washing three times with buffer medium (pH 7.35). Approximately the upper and lower quarters of the packed cells were removed, each resuspended in buffer medium containing L-1-14Cglucose, and centrifuged after 3 min. Radioactivity in cells and buffer medium was then measured in a liquid scintillation counter. Details of the materials and general methods used will be published elsewhere (K.D. Neame and C.A. Homewood, unpublished). The possibility that the pooled blood contained erythrocytes from uninfected or poorly infected animals was avoided by examining blood from each animal, and rejecting any animals in which the percentage of erythrocytes parasitised was particularly low for the group.

The uptake of the labelled L-glucose by the erythrocytes is shown in Fig. 1. Uptake has been expressed in terms of the distribution space in the centrifuged packed cells, which included residual buffer medium. The inulin space of packed erythrocytes is therefore also given, and the L-glucose space of erythrocytes from normal animals is included in the figure for comparison. It can be seen that the parasite-rich fraction of cells (top layer of packed cells) showed a consistently higher L-glucose space than did the parasite-poor fraction (bottom layer), and that the size of the L-glucose space was proportional to the percentage of erythrocytes which were parasitised.

These findings strongly suggest that only those cells which contain parasites become readily permeable to L-glucose, and that the change in permeability thus appears to be a direct effect of the parasite on the cell in which it is contained, and is not merely a generalised effect on all erythrocytes. This is supported by the further finding that incubation (at 37° C for 1 h) of normal erythrocytes with plasma from infected mice had no effect on their subsequent permeability to L-glucose.

This increase in the permeability of the parasitised erythrocyte may be a striking example of the means by which a parasite, in addition to adapting itself to the host, might also adapt the host cell to its own requirements by allowing the entry into the erythrocyte of certain nutrients normally excluded from the cell.

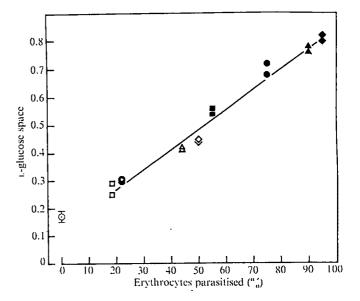


Fig. 1 Relationship between uptake of L-glucose by erythrocytes and percentage of erythrocytes parasitised. Erythrocytes from mice infected with P. berghei were fractionated as described in the text. Each group of four symbols of similar shape gives values from a single pooled sample of blood; the upper pair (closed symbols) represent duplicate samples from the top layer of centrifuged cells, and the lower pair (open symbols) represent duplicate samples from the bottom layer. Inulin space of packed cells: normal mice,  $0.14 \pm 0.02$  s.d., n = 6; mice with about half the erythrocytes parasitised,  $0.19 \pm 0.02$  s.d., n = 8.  $\odot$ , L-glucose space of erythrocytes from uninfected mice (mean  $\pm$ s.d., n = 6).

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#### Tyrosine-dependent increase of tyrosine hydroxylase in neuroblastoma cells

Tyrosine hydroxylase (tyrosine-3-monooxygenase), presumably the rate-limiting enzyme in the biosynthesis of the adrenergic transmitters dopamine and noradrenaline<sup>1,2</sup>, catalyses the hydroxylation of both phenylalanine and tyrosine3.4. This property has been exploited for the selection of adrenergic-like mouse neuroblastoma cells5, which have high tyrosine hydroxylase activity and can grow in the absence of tyrosine because they can convert sufficient phenylalanine to tyrosine for cell growth. N1E-115 is such a neuroblastoma clone<sup>5,6</sup> and we have now found that, when the amount of tyrosine in the medium is increased, there is an increase in the amount of tyrosine hydroxylase in N1E-115 cells. Modification of the amount of this ratelimiting enzyme by its substrate concentration may play a fundamental role in the regulation of the biosynthesis of catecholamines.

NIE-115 cells (provided by Dr Marshall Nirenberg) were grown as monolayer cultures in polystyrene Petri dishes, 150 mm in diameter (Falcon), at 37° C in a humidified atmosphere of 10% CO<sub>2</sub> and 90% air. Cells were grown as before in the Dulbecco-Vogt modification of Eagle's medium (DNEM, Gibco, catalogue No. H-21) containing 4.5 g of dextrose per litre, 0.42 mM tyrosine and phenylalanine, no Na pyruvate and 5% foetal calf serum. Cells were maintained in stationary phase for approximately 1 week and fed a mixture of 3 parts F-14 medium8 and 1 part DMEM which contained a final concentration of 0.16 mM tyrosine and 0.21 mM phenylalanine. During experimental procedures, cells were maintained in F-14 medium containing 0.12 mM phenylalanine, various concentrations of tyrosine and 2% dialysed foetal calf serum. After experimental incubations monolayers were washed three times with an isotonic salt solution and cells were collected by scraping and washing with approximately 1 ml of 0.05 M potassium phosphate buffer (pH 6.8) containing 8% sucrose. The recovered suspension was sonicated and centrifuged at 30,000g for 30 min. The clear extract that we used contained more than 90% of the total tyrosine hydroxylase activity. Tyrosine hydroxylase activity was measured by the tritium release method9. The standard assay was carried out in 0.50 ml that contained: 50 µmol potassium phosphate (final pH 6.1); 25 nmol L-tyrosine containing 800,000 c.p.m. of 3,5-3H-L-tyrosine (original specific

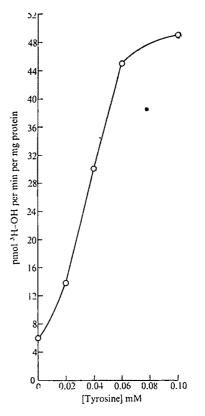


Fig. 1 Effect of increasing levels of tyrosine in the culture media on tyrosine hydroxylase activity in N1E-115 neuroblastoma cell extracts.

activity 1.0 Ci mmol<sup>-1</sup>) (Amersham-Searle) purified before use<sup>10</sup>. 20 μmol β-mercaptoethanol, 2,000 U catalase (Boehringer-Mannheim), 0.50 µmol 6-methyltetrahydropterin (Calbiochem) and the tyrosine hydroxylase-containing extract to be assayed (0.3-0.7 mg protein per assay). Incubations were carried out at  $37^{\circ}$  C for 10 min and stopped with 0.5 ml of 10% trichloroacetic acid. Protein was determined by the method of Lowry et al. with bovine serum albumin as a standard11. The tyrosine hydroxylase activity from this cell line has been reported to be higher than the activity described here12. Several variables may account for this difference, for example: (1) previous use of 10% undialysed foetal calf serum, (2) growth of cells to a greater degree of differentiation before and (3) use of 500  $\mu M$  tyrosine in the earlier assay system compared with the use of 50  $\mu M$  tyrosine in the present assay system. Specific tyrosine hydroxylase antiserum was produced in sheep as before<sup>13</sup>. A crude γ-globulin fraction was obtained by fractionating control and immune sera by the addition of saturated ammonium sulphate at pH 6.8 until 25% saturation was achieved. The pellet was collected by centrifugation and resuspended in the original volume of serum with 0.02 M potassium phosphate and 0.3 M glycine buffer, pH 6.8. The 0-25% ammonium sulphate fractionation was repeated and the second pellet was suspended in the same volume of phosphate-glycine buffer.

When N1E-115 cells were incubated for 24 h in media containing tyrosine, the activity of tyrosine hydroxylase increased eightfold as tyrosine concentration increased from 0 to 0.10 mM (Fig. 1). In other experiments (not shown) the increase in tyrosine hydroxylase activity levelled off at concentrations of 0.10-0.30 mM tyrosine. Results were similar with another adrenergic-like neuroblastoma cell line N-TD65. During the 24 h exposures to various concentrations of tyrosine, total cellular protein per dish did not vary more than 15%. Total and specific tyrosine hydroxylase activity in cells maintained without tyrosine for 24 h and then exposed to 0.10 mM tyrosine for 0, 1, 4, 9, 13 and 24 h, increased in a time-dependent manner (Fig. 2). After 24 h this enzyme activity had increased threefold. It is not apparent why a larger increase was not observed.

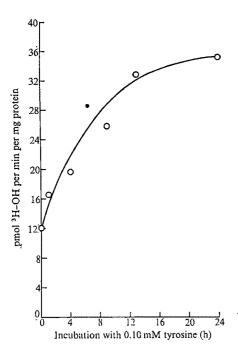


Fig. 2 Increase in tyrosine hydroxylase activity in N1E-115 neuroblastoma cell extracts as a function of time of incubation in media containing 0.10 mM tyrosine. The cells were first maintained for 24 h in F-14 medium containing 0.12 mM phenylalanine, 2% dialysed foetal calf serum and no tyrosine. During the experiment the cells were maintained in the same medium with 0.10 mM tyrosine.

The increase in tyrosine hydroxylase activity in response to tyrosine in the medium could have been due to the synthesis of new enzyme molecules, or the activation of existing enzyme molecules. We have investigated this question by titrating N1E-115 extracts with specific anti-tyrosine hydroxylase  $\gamma$ -globulin. Some results are presented in Fig. 3, which show that inhibition of the higher level of tyrosine hydroxylase activity required proportionally more antibody. Extrapolation of the titration data to the abscissa gives equivalance points which indicate that 1.66 times as much antibody would be required to inhibit totally tyrosine hydroxylase activity of cells grown in 0.10 mM tyrosine as of those grown in 0.04 mM tyrosine.

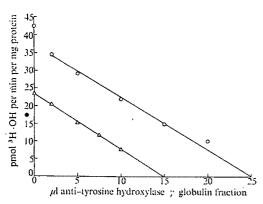


Fig. 3 Titration of tyrosine hydroxylase activity from N1E-115 neuroblastoma cell extracts with specific anti-tyrosine hydroxylase y globulin. Extracts were prepared from cells maintained for 24 h in F-14 mdeium containing 0.12 mM phenylalanine, 2% dialysed foetal calf serum and 0.04 mM ( $\triangle$ ) or 0.10 mM ( $\bigcirc$ ) tyrosine. Each tube contained an equivalent volume of extract to be assayed (0.64 mg protein per tube from the 0.04 mM tyrosine dish extracts, and 0.72 mg protein per tube from the 0.10 mM tyrosine dish extracts). So that each sample would contain the same total amount of  $\gamma$  globuling control  $\gamma$ -globulin fraction was added to bring them all to the same volume and the tubes containing no anti-tyrosine hydroxylase  $\gamma$  globulin contained only control γ globulin. The tubes were incubated at 25° C for 10 min, then at 0° C for 4 h and then assayed for tyrosine hydroxylase activity.

Extracts of the former cells contained 1.60 times as much enzyme activity as did extracts of the latter. Titration of extracts from cells grown in media containing other concentrations of tyrosine showed similar proportionality between enzyme activity and the amount of antibody necessary to achieve a given degree of inhibition. Thus, the increases in tyrosine hydroxylase activity are due to an increase in tyrosine hydroxylase molecules.

Increases in catechol and catecholamine synthesis in nerve cells in response to increasing levels of external tyrosine1,14,15 have been interpreted to imply that, at least under some conditions, uptake of tyrosine may be a rate-limiting step in catecholamine biosynthesis. Our results suggest that these observations also reflect the ability of tyrosine to increase the amount of cellular tyrosine hydroxylase. We have not yet determined whether the observed increase in tyrosine hydroxylase protein is due to increased synthesis or decreased degradation of the enzyme. Previous studies of increased enzyme activity in mammalian tissues caused by substrates have shown that the substrate can protect the enzyme against inactivation or degradation, for example, L-tryptophan on tryptophan oxygenase16.17, and thymidine on thymidylate kinase18. Further studies are necessary to confirm or disprove the possibility that increased extracellular tyrosine elicits an increase in neuroblastoma tyrosine hydroxylase protein by protecting the enzyme against degradation.

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# Lymphocyte cytotoxicity to isolated hepatocytes in chronic active hepatitis

CHRONIC active hepatitis is the term used to describe a chronic progressive liver disease with superimposed episodes of activity, which is characterised histologically by a dense mononuclear cell infiltrate in the portal tracts and piecemeal necrosis of periportal hepatocytes. Indirect evidence suggests that this may be an autoimmune disease. Antibodies reacting with smooth muscle, nuclei or mitochondria are present in the majority of patients¹ and there is also evidence of a cell-mediated autoimmune reaction as shown by the finding of inhibition of leukocyte migration by a human liverspecific lipopfotein (LSP)². This macrolipoprotein, when injected repeatedly into rabbits, will induce chronic aggressive hepatitis, and is thought to be a normal constituent of the hepatocyte plasma membrane³.

Work using antibody assays and immunofluorescence studies has demonstrated complete organ but incomplete species specificity, with cross reaction between the rabbit and human protein<sup>3</sup>. Here we describe the use of isolated rabbit hepatocytes for investigating the cytotoxicity of lymphocytes from patients with chronic active hepatitis, or other possible autoimmune liver diseases, and the role of the liver-specific protein as the principal antigen involved.

Twenty-two patients with chronic active hepatitis were studied. Tests were carried out in six patients with primary biliary cirrhosis, four with primary hepatocellular carcinoma, five with inactive alcoholic cirrhosis, one with idiopathic haemochromatosis and one with obstructive jaundice, and also in eleven healthy controls. Isolated rabbit hepatocytes were seeded into microtest culture plates (Falcon 3034) to achieve a final concentration of approximately 70 cells per well, and cultures were gassed with a 95% O<sub>2</sub>: 5% CO<sub>2</sub> mixture and incubated for 24 h at 37° C.

About 18 h after the establishment of the cultures, the supernatant was aspirated from each well and replaced with 10  $\mu$ l of lymphocyte suspension, the concentration of lymphocytes being adjusted to produce a lymphocyte to target cell (hepatocyte) ratio of 400: 1. After incubation at 37° C without agitation for 48 h the plates were inverted for a further 2 h and then carefully rinsed in medium 199. The number of hepatocytes remaining in each well was counted at ×60 magnification using a graticule eyepiece. Each experimental procedure was performed with at least 10 replicate wells, and each plate had its own group of 20 control wells. The difference between the mean number of cells in control and test wells, when expressed as a fraction of the former, gave the percentage cytotoxicity. Negative values have been represented as showing zero cytotoxicity. The range of cytotoxicity in control subjects so closely approximated to a normal distribution (mean 2.9%; s.d. 14.4%) that two standard deviations from the mean was thought to provide a reasonable estimate of the upper limit of the normal range.

Significant cytotoxicity was demonstrated in 20 (90%) of the patients with chronic active hepatitis, and was also present in two of the six patients with primary biliary cirrhosis (Fig. 1). In the 11 patients with other forms of chronic liver disease the results were similar to the controls, none showing significant cytotoxicity. Four of the 22 patients with chronic active hepatitis were untreated at the time of study and all showed significant cytotoxicity (56%, 70%, 89% and 84%). The remaining 18 cases were receiving various combinations of immunosuppressive drugs, and had shown improvement in biochemical liver function, but in only two did the result of the cytotoxicity test fall within the normal range. Serial studies in the four untreated patients, however, showed a significant reduction in the cytotoxicity following treatment with oral prednisone from a mean of 75-30% (t=3.2, P<0.05).

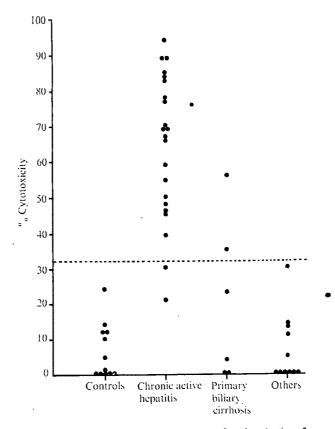


Fig. 1 Percentage loss of hepatocytes after incubation for 48 h with lymphocytes from patients with the various chronic liver diseases shown. The horizontal dotted line indicates the upper limit of the normal range (2 s.d. above the mean of the results in the controls). Lymphocytes were prepared from heparinised blood by dextran sedimentation and purified by cotton wool filtration<sup>4</sup> and centrifugation over a Ficoll/triosil gradient<sup>5</sup>. The final preparation contained, on average, 98% lymphocytes and 2°, monocytes. Three-month-old New Zealand White rabbits were used for the preparation of isolated hepatocytes. The liver was cut into small pieces and then incubated at 37° C for 12 h in RPMI 1640 containing foetal calf serum 10°, hyaluronidase 0.1°, collagenase 0.01%, DNase 0.005%, 1 M HEPES 2.3°, collagenase 0.01%, DNase 0.005%, 1 M HEPES dijusted to a pH of 7.35 in an atmosphere of 95°, O<sub>2</sub>:5% CO<sub>2</sub>. Short term tissue cultures were then prepared, the culture medium being identical to that used above but containing no enzymes. Indirect immunofluorescence using antisera obtained by immunisation of guinea pigs with the liver-specific cell surface antigen were used to verify that the cells were hepatocytes.

If this cytotoxicity is a consequence of specific sensitisation to LSP, the addition of excess of this lipoprotein should block the reaction. The mean cytotoxicity of four patients with chronic active hepatitis, when lymphocytes alone were added to the incubation chambers, was  $53.6 \pm 5.7\%$ . The addition of either human or rabbit LSP (0.5 µg per well), prepared from normal rabbit tissue and human organs<sup>6</sup>, together with the lymphocytes produced a highly significant reduction in cytotoxicity,  $5.3\pm3.0\%$  and  $5.5\pm5.5\%$  respectively. To confirm the specificity of this blocking reaction, a human kidney protein fraction, prepared in an identical manner to LSP, was added with the lymphocytes. The mean cytotoxicity in these conditions was almost identical to that obtained with lymphocytes alone (56.4  $\pm$  6.8). The addition of an antibody to LSP raised in guinea pigs, which by immunofluorescence was shown to coat the hepatocytes. completely blocked the cytotoxic reaction.

The blocking experiments clearly implicate sensitisation to human LSP in chronic active hepatitis as the important factor in the production of the lymphocyte-mediated hepatocyte injury. Indeed, preliminary analysis has shown a good

correlation between sensitisation to LSP as detected by the leukocyte migration test and the degree of lymphocyte cytotoxicity to isolated hepatocytes. Such sensitisation might be initiated by structural changes in the membrane lipoprotein following virus or drug-induced liver damage, but little information is available concerning either this aspect of pathogenesis or the aberrations in immunological control systems which may determine persistence of autoimmunisation.

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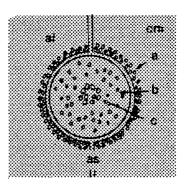
# Intramembraneous changes on cationophore-triggered exocvtosis in *Paramecium*

Secretory vesicles ('trichocysts') of Paramecium contain proteins1 which are discharged by exocytosis. In P. aurelia (mating type VIII), trichocysts are firmly attached to the cell membrane before the onset of exocytosis, and membrane-intercalated granules occur at these contact sites (Fig. 1); these granules are arranged in a regular pattern and have been described previously as 'b-' and 'c-type granules'2. They are surrounded by a ring of 'a-type granules' which connect the cell membrane to the membrane of the alveolar sacs in the region where the latter are penetrated by the trichocysts<sup>2,3</sup>. When the trichocysts are expelled, the cell membrane is penetrated within the ring of a-type granules and the trichocyst membrane fuses temporarily with the cell membrane<sup>2</sup>. The present study takes advantage of several circumstances: (i) cells could be frozen without prefixation or cryoprotectants; (ii) exocytosis triggering involves only the final step of membrane fusion; (iii) the regular 'landmarks' in the plasmalemma serve to pinpoint intramembraneous changes occurring during exocytosis.

Exocytosis, monitored using the phase microscope, was triggered by enhancing transmembraneous ion flux via cationophores, that is, small molecules incorporated spontaneously into membranes. Intramembraneous morphological changes were analysed by freeze-fracture. Ionophores used were X-537 A (Hoffman-LaRoche), which complexes reversibly with mono- and bivalent cations, or A 23187 (E. Lilly), which complexes with bivalent cations only4. Exocytosis is connected in various cell types with Ca2+ influx5; the same ionophores also trigger exocytosis in

various other systems if Ca2+ is added6-8. In untreated paramedia, spontaneous trichocyst discharge is rarely observed. Addition of Ca<sup>2+</sup> to Paramecium cultures does not provoke discharge in the absence of ionophores. The Ca2+ content of the medium (0.4 mM), determined by atomic absorption with a Jarrel Ash unit, is much higher than the concentration thought to occur within Paramecium cells (10<sup>-4</sup> mM) (ref. 9) and is sufficient to trigger some exocytosis on addition of ionophores alone. Chelation of extracellular Ca<sup>2+</sup> with 2.5 mM EDTA, which only gradually immobilised the animals, prevented trichocyst exocytosis on addition of 0.10 mM X-537 A. With appropriate concentrations of ionophore A 23187 (0.07 mM) and Ca2+ (17 mM), practically all paramecia discharged almost all their trichocysts within 20 min before gross morphological changes were detectable on the cells or their trichocysts, although the latter extend to several times their resting length during expulsion. Preliminary attempts to localise intracellular Ca2+ suggested that a Ca2+ shift into the trichocysts precedes exocytosis (H.P., unpublished). Trypan blue did not penetrate into a given cell before exocytosis of most trichocysts was completed, which excludes a bypass of Ca2+ influx through gross membrane leaks.

During freeze-fracture the plasmalemma splits along a central plane in Paramecium<sup>2</sup> as in several other membranes<sup>10</sup>. The fracture face attached to the cytoplasm is called A face: the complementary view of the split outer half represents the B face. Cell membranes of controls exhibit on their A face b- and c-type granules at the sites of trichocyst attachments; c-type granules are especially visible as  $\sim 800$  Å clusters on the B face (Fig. 2a); the attachment sites of trichocysts are enclosed by a-type rings consisting of double (or occasionally single) rows of granules on the A face; corresponding holes occur on the B face. Since one cannot recognise on a replica of a freezecleaved cell membrane whether exocytosis has occurred,



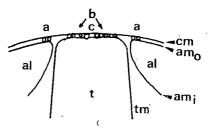


Fig. 1 a, a-Type ring of membrane-intercalated granules, connecting cell membrane and alveolar membranes; al, alveolar cavities (interrupted at sites of trichocysts); ami, (amo) inner (outer) membrane of alveolar cavities; as, alveolar septa; b, diffuse aggregates of membrane-intercalated particles (located within a-type rings) at contact sites between cell membrane and trichocyst membrane; c, ~800 Å large patch of membrane-intercalated particles at the centre of the site of contact between trichocyst membrane and cell membrane; cm, cell membrane (dotted); t, trichocyst; tm trichocyst membrane.

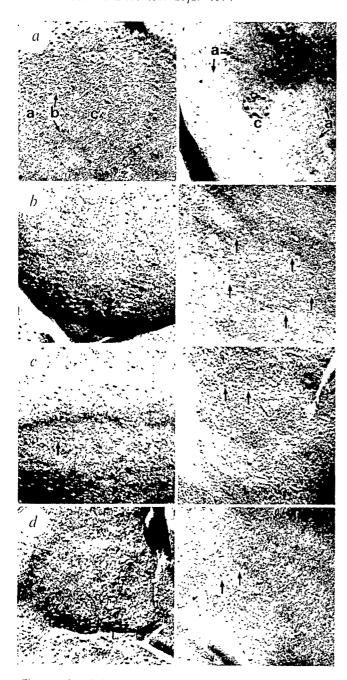


Fig. 2 A face (left) and B face (right) of the split cell membrane after freeze-cleaving. Controls (stage a) are compared with different stages of morphological changes after triggering exocytosis (stages b-d) obtained by adding 0.07 mM ionophore A 23187 and 17 mM  $Ca^{2+}$  for 15–18 min. Stages b-d may be encountered in close vicinity within one animal; c-type granules invariably disappeared after exocytosis. Stage d could be identified on the basis of the regular geometrical arrangement of trichocyst attachments on the body surface². All samples were frozen in Freon 12 without prefixation or cryoprotectants, cut at  $-100^{\circ}$  C and shadowed with platinum-carbon in a Balzers BA 360 M freeze-etching unit. Micrographs are mounted with white shadows, the shadow casting direction is from bottom to top. a-Type rings and their remnants (arrows) appear as granules on the A face and as corresponding holes (faintly visible) on the B face. ( $\times$  100,000.)

conditions were chosen, where complete triggering of exocytosis was achieved. After exocytosis a-type rings persisted in various degrees of decomposition on A and B faces (Fig. 2b-d, Table 1) and could therefore be used as 'landmarks' to locate the sites where exocytosis had taken place; furthermore, these sites are located at predictable points of the body surface<sup>2</sup>. After exocytosis, c-type granules almost

invariably disappeared from the B face (Fig. 2b-d, right); b-type granules also seem to be dispersed, although they are arranged diffusely, even in controls. a-Type rings often persist as fragments, although the number of non-oriented particles seems greater in regions where a-type rings undergo decay (Fig. 2c, left). This suggests that granules are dispersed by lateral movement and that after exocytosis the cell membrane is resealed by lateral diffusion of membrane (lipid) constituents; this latter assumption is further supported by the observation that a-type rings are smaller in triggered cells than in controls (Table 1). Ultrathin sections show that the dispersion of a-type rings in the period following massive trichocyst discharge could be caused by a gradual detachment of the plasmalemma after exocytosis and membrane resealing have been completed. Thus, the most specific change related directly to exocytosis is the disappearance of c-type granule aggregates. Since these are present within most of the a-type rings in unstimulated, normal cells, their occurrence cannot be explained by membrane fusion during exocytosis<sup>11,12</sup>; rather, in Paramecium they represent stable structural elements at the sites where trichocysts are attached permanently to the plasmalemma prior to the membrane fusion occurring during exocytosis.

The intracellular membrane-to-membrane attachment sites2 of types a-c in normal Paramecium do not represent selective permeability sites which would be directly analogous to gap junctions13. This follows from studies with electron microscopic tracers such as La(OH)3; all membraneto-membrane attachment sites of Paramecium are also impermeable to (2% w/v) microperoxidases (haemoligopeptides, molecular weight approximately 1,700-1,900), applied to living Paramecium over several days (H.P., unpublished). If, during spontaneous exocytosis, the membrane-intercalated particles function in analogy to ionophores (not resolvable in replicas) the particles would have to be capable of very rapid permeability changes specifically for Ca2+. Other functional aspects of membrane-intercalated particles, such as the possible role of lipid constituents in membrane fusion<sup>14</sup> and Ca<sup>2+</sup>-dependent ATPases<sup>4</sup> possibly being involved in exocytosis also have to be considered.

Table 1 Morphological changes of a-type rings in freeze-fractured replicas after exocytosis\*

•				
Treatment		ition stages Frequency‡		(nm)§ Large diameter
Controls	b - d	97 3	254±43	$313 \pm 36$
15-18 min 0.07 mM ionophore A 23187 - 17 mM Ca <sup>2+</sup>	+ b c d	$ \begin{array}{c} 0 \\ \sim 28 \\ \sim 26 \\ \sim 46 \end{array} $	195±38	243±29

\*Samples were processed for freeze-cleaving as described in Fig. 2. †Stages of decomposition (a-d) are as defined in Fig. 2.

Numbers of a-type rings counted were 59 for controls and 39 after exocytosis.

\$Values  $\pm$  s.d. Since trichocysts do not occur over the whole body surface, only those regions surrounded by intact or clearly visible remnants of a-type rings were evaluated; frequency values show that in such regions a-type rings are only rarely missing in controls: therefore, values determined for stage d after exocytosis are necessarily minimum estimates.

As discharge is very rapid, time sequence studies of morphological changes could not be performed. The occurrence of similar granular membrane specialisations has been reported not only in *Tetrahymena*<sup>11,12</sup> and *Paramecium*<sup>2</sup> but also in connection with transendothelial movement of cytopempsis vesicles<sup>15,16</sup>. In contrast, no such intramembraneous differentiations were observed in various gland cells<sup>17–20</sup>, stimulated or unstimulated. In these cases, membrane-to-membrane attachment might possibly have escaped detec-

tion, either because it is an ephemeral event or because of the pretreatments commonly used before freezing. Non-cryoprotected Paramecium, however, cationophoremediated transmembraneous Ca2+ influx provokes trichocyst exceytosis, accompanied by specific changes of structural subunits preforms within the membrane portions involved.

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## Acute hepatic injury by vinyl chloride in rats pretreated with phenobarbital

LITTLE is known about the biological effects of vinyl chloride monomer (VCM) despite its wide industrial use. In 1973, thousands of workers were involved in producing, processing and moulding VCM into 15 billion pounds of its polymer (PVC)1. Angiosarcoma, a rare liver cancer, has recently been linked to occupational exposure in man2 and long term inhalation of low doses in animals3. Occupational vinyl chloride exposure has also been associated with increased incidence of hepatic cirrhosis, splenomegaly, decreased bromosulphalein clearance from blood and acro-osteolysis4.5. Reports of acute hepatic injury after vinyl chloride inhalation are limited and the findings relatively nonspecific<sup>6,7</sup>.

VCM, readily reactive in free radical-mediated industrial polymerisations, may interact with liver mixed function oxidase system (MFOS), an intracellular enzyme complex located within the membranes of the endoplasmic reticulum. This system is known to activate other halogenated hydrocarbons into hepatotoxins\*. Therefore, we set up an experiment to determine whether the potential of VCM for liver injury was enhanced in animals pretreated with phenobarbital (PBT), an inducer of certain enzymes of the MFOS.

Male Holtzman rats (300 g) were exposed once to air, or air containing 0.5, 5 or 10% VCM for 6 h or to air alone or air containing 0.5 or 5% VCM for 6 h on each of 5 consecutive days in a dynamic inhalation chamber previously described. VCM was obtained from Matheson Gas Corporation. Rats were given untreated drinking water regularly or they were pretreated with sodium phenobarbital (0.1%) in the drinking water beginning 7 d before exposure to VCM. This dose, which corresponds to 100 mg kg<sup>-1</sup> d<sup>-1</sup>, is sufficient to double both cytochrome *P*-450 content and oxidative N-demethylase activity<sup>10-11</sup>. Four experimental groups were defined: (1) exposed to air and not pretreated; (2) exposed to VCM and not pretreated; (3) exposed to air and pretreated with PBT; and (4) exposed to VCM and pretreated with PBT. Animals were killed 24 h after the single exposure, or immediately after the fifth. Blood was collected to measure serum enzyme activity indicative of liver damage". The liver tissue was prepared for histological examination by perfusion fixation through the portal vein with buffered 1% glutaraldehyde. Selected blocks were post-fixed with OsO<sub>4</sub>. Thin sections of Epon-embedded liver were examined by electron microscopy<sup>11</sup>.

Effect of phenobarbital pretreatment on serum enzyme activities 24 h after onset of a single 6-h VCM exposure

Exposure*	Pretreatment *			
·	None	PBT		
	AKT: SDH§	AKT: SDH§		
Air (5)	0.27 : 0.02   7.0 : 1.7	0.27 · 0.05 8.0 <u></u> 0.6		
0.5% VCM† (3)	$0.28 \cdot 0.01  4.0 \cdot 0.4$	0.43:_0.15 16.8:_10.0		
5% VCM† (2)	(0.17,0.14) (6.7,5.6)	(3.41, 7.88) (160, 256)		
10% VCM (4)	0.42 -0.10 25.1 - 9.8			

\*Numbers in parentheses are number of animals.

†VCM concentration verified by gas chromatography.

Alanine-u-ketoglutarate transaminase activity: mg pyruvate ml-1 h-1. Sorbitol dehydrogenase activity; units ml-1 min-1

Expressed as mean 's.e. or as range.

The biochemical data from these experiments are shown in Table 1. In this table, both non-pretreated and PBT-pretreated animals exposed to air alone had no rise in levels of serum alanine-α-ketoglutarate transaminase (EC 2.6.1.2, AKT) or sorbitol dehydrogenase (SDH), another cytoplasmic liver enzyme whose appearance in serum correlates with liver injury<sup>12,13</sup>. We have previously shown this enzyme to be highly specific for several types of liver injury<sup>14</sup>.

In non-pretreated groups, exposure to 0.5% or 5% VCM for a single 6-h period did not cause a substantial rise in either serum AKT or SDH activity. Only after exposure to 10% VCM was there a slight increase in either parameter of hepatoxic response. In these animals, livers were histologically normal at the lower dose levels of VCM (Fig. 1a), and centrolobular hepatocellular vacuolisation was noted only in the group exposed to 10% VCM. During exposure to this higher dose level, animals appeared to be anaesthetised.

By contrast, PBT pretreatment for 7 d caused a marked enhancement of injury at the 5% level of exposure. Rats exposed to 0.5% were without apparent injury, determined biochemically. In the PBT-pretreated rats exposed to 5% VCM a clear morphological response was observed with striking vacuolisation of centrolobular parenchymal cells (Fig. 1b). There was also focal necrosis of mid-zonal parenchyma which became confluent towards the dorsal aspect of the liver. This vacuolated change corresponds to dilation of the rough endoplasmic reticulum as seen by electron microscopy. Smooth

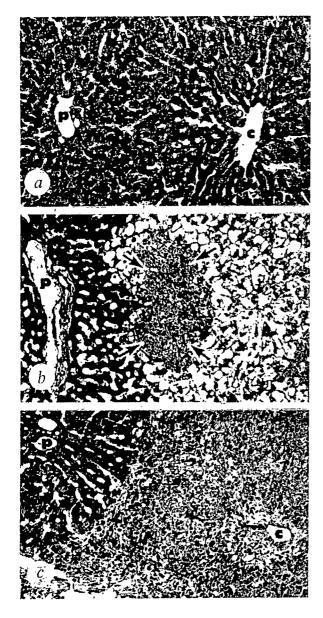


Fig. 1 Liver: portal areas (p) on left; central veins (c) on right. a, Non-pretreated rat 24 h after 6-h exposure to 5% VCM. b, PBT rat 24 h after 6-h exposure to 5% VCM. There is extensive vacuolisation of centrolobular parenchyma on right and a focus of midzonal necrosis at centre (arrows). c, PBT rat immediately after last of five 6-h exposures to 5% VCM at 24-h intervals. Necrotic centrolobular parenchyma on the right has been removed, remaining connective tissue stroma is infiltrated by macrophages. Vacuolated parenchymal cells are not seen. Stain: haematoxylin and eosin. < 150.

endoplasmic reticulum in involved cells form tubular snarls with foci of increased electron opacity suggesting of membrane denaturation11. In one of four PBT-pretreated animals exposed to 0.5% VCM, such vacuolisation, but not necrosis, was also observed. As noted previously, centrolobular vacuolisation was seen only in livers of non-pretreated rats exposed to 10%

The serum from PBT-pretreated rats killed after the last exposure to 5% VCM following 5 consecutive days of exposure did not contain raised activities of either serum AKT or SDH. This suggests that acute hepatotoxic injury was not cumulative, or that previous exposure to VCM blocked the biochemical response which followed re-exposure at short intervals<sup>15</sup>. Preliminary time course studies showed that increases in serum enzyme activities reached a maximum 24 h after the start of VCM exposure. Thus, injury from day 4 of exposure should have been shown on day 5 when rats were killed. Instead of a

biochemical lesion, Fig. 1c shows that livers of PBT-pretreate animals successively exposed to 5% VCM contained broa areas of parenchymal loss and stromal collapse most prominer towards the dorsal aspect of the liver. These bands correspon in distribution to the bands of confluent necrosis seen after single exposure. Although these animals were killed 24 h afte the fourth exposure, and immediately following the fifth, the. was neither vacuolisation nor acute focal necrosis finding indicative of recurrent acute injury. Results of the multiple 5' VCM exposure suggest that PBT-pretreated animals are protected against recurrent acute hepatocellular injury from dai VCM exposure by previous injury-inducing VCM exposur Livers from non-pretreated animals similarly exposed up to 5° VCM on 5 consecutive days were indistinguishable from nor pretreated animals exposed to air.

The hepatotoxicity of vinyl chloride may be caused by mechanism similar to that of other non-symmetrically chloric ated ethylenes such as trichloroethylene (TCE). Inductic of MFOS enzymes by pretreatment with PBT or 3-methy cholanthrene enhances the hepatotoxicity of TCF16, possibl through an increased rate and/or altered route of metabolism1 Interestingly, after an initial exposure to TCE, the in viv metabolism following subsequent exposures is not reduced but the ratio of secondary metabolites found in the urin changes18. From our data, the initial exposure to VCM seem to protect against acute injury on re-exposure for at least 5 c Perhaps, this initial exposure alters the pathway of activation of VCM in a manner not unlike that proposed for CCl<sub>1</sub> (ref. 15 which destroys MFOS enzymes involved in its metabolic activa tion to a hepatotoxin. Like TCE19, the metabolism of VCM ma proceed through an epoxide intermediate. Activation to reactive electrophile20 is also in keeping with the tumorigenia potential of VCM. Experiments to define the interactions o VCM and the MFOS are underway.

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## Application of β-D-glucuronides and glucose together suggests a new direction for cancer chemotherapy

KNOWN anti-cancer agents are unselective, attacking not only tumour cells but also other rapidly proliferating cells such as lymphocytes. Any difference between tumour and normal cells which might provide a basis for specific chemotherapy has therefore aroused interest. One such difference is the lower pHvalue of tumour tissue (about 6) compared with most normal tissue (about 7.3) in the presence of glucose<sup>1,2</sup>.

The lysosomal enzyme  $\beta$ -glucuronidase has a  $\rho H$  optimum of 5.2 (ref. 3). We report here that the glucuronidase activity of tumours is selectively enhanced by previous administration of glucose, presumably because glucose selectively lowers the pH of tumour cells. Since glucuronides are biologically inactive4.5, our observation would suggest the possibility of designing drugs which are preferentially activated in tumour cells.

Tumour cells  $(5 \times 10^5)$ , counted with a haemocytometer, were implanted subcutaneously in the neck of a male Hauben rat (6 weeks old). The spindle-shaped sarcoma was induced in 1968 using methylnitrosourea and repeatedly transplanted<sup>6</sup>. After 4 weeks the rat bearing the tumour was treated six times with glucose (2 g kg<sup>-1</sup>) (50% in phosphate buffer pH 7.3) intraperitoneally at 40 min intervals. Two hours after commencing treatment, β-hydroxyquinoline-β-D-glucuronide (Light, Colnbrook, UK, 500 mg kg<sup>-1</sup>, suspended in the glucose solution) was applied intraperitoneally. Six hours after beginning the experiment, the animal was killed, using ether. The tumour and surrounding muscle were removed, and 1 g of each tissue homogenised in 5 ml distilled water in a Potter homogeniser. The homogenates were then continually extracted with boiling ether for 2 h, the ether extracts dried over Na<sub>2</sub>SO<sub>4</sub>, evaporated to approximately 0.5 ml and the residue investigated by thin layer chromatography (TLC) (Silufol UV<sub>254</sub>, Kavalier, CSSR). 8-Hydroxyguinoline was present in the tumour extract, but not in the muscle extract ( $R_F$  0.39 in benzene/acetone (1:1) and 0.143 in benzene/methanol (95:5), identical with those of the control sample). 8-Hydroxyquinoline was detected using its UV-extinction property.

	Table 1 Ex	tinction o	f tissue homogenates at 480 nm
	Tumour	Muscle	
E*480	0.254	0.073	†Treated with 8-hydroxyquinoline-β-D-
E*480	0.078	0.083	glucuronide and glucose †Treated with 8-hydroxyquinoline-β-D- glucuronide
E*480	0.084	0.082	†Untreated for control

E\*480 of a solution of 0.1 mg 8-hydroxyquinoline in 1 ml 2 N NaOH + 2 ml reagent = 0.375.

†All rats were approximately the same weight (200-220 g) and had tumours of approximately the same size (1 g).

A second tumour-bearing rat was similarly treated; the ether solution of the homogenates was totally evaporated, and the residue treated with 1 ml 2 N NaOH and 2 ml of a reagent containing diazotated p-nitroaniline. The extinction was measured at 480 nm (Table 1).

A third rat was treated with 8-hydroxyquinoline-β-D-glucuronide (500 mg kg<sup>-1</sup>, suspended in 2.5 ml phosphate: buffer pH 7.3), but without glucose. The extracts were prepared as described above and investigated using TLC and photometry. 8-Hydroxyquinoline was not detected in the tumour or in the muscle extract (Table 1).

Two more rats, each bearing a neck tumour, were treated with 2-naphthyl- $\beta$ -D-glucururonide sodium salt (330 mg kg<sup>-1</sup> Koch-Light Colnbrook), together with glucose (12 g kg<sup>-1</sup>), and two other rats with the glucuronide without glucose. We could only detect 2-naphthol in the tumour extracts of the rats treated with both 2-naphthyl-β-p-glucuronide and glucose. Barely visible traces of 2-naphthol were detected in the other tissue homogenates. The extracts were investigated using TLC (silica gel), R<sub>1</sub> 0.31 in benzene/methanol (95:5) and 0.61 in benzenedioxan-acetic acid (90:25:4), identical to those of the control sample. Diazotated benzidine8 was used for detection.

The 2-naphthole content in the tumour tissue was also measured quantitatively. The residue of the extracts was dissolved in 2 ml ethanol and 0.02 ml of a solution containing diazotated benzidine (0.5 g benzidine in 0.14 ml concentrated HCl and 98.6 ml water mixed with 100 ml 10% NaNO<sub>2</sub> in water) were added. The extinction was measured at 415 nm against a solution of 0.02 ml reagent and 2 ml C<sub>2</sub>H<sub>5</sub>OH (Table 2).

	Table 2 Ex	tinction o	of tissue homogenates at 415 nm
	Tumour	Muscle	
E*115	0.372	0.036	†Treated with 2-naphthyl-β-D-glucuro- nide and glucose
E*113	0.028	0.031	†Treated only with 2-naphthyl-β-D-glucuronide
E*115	0.022	0.021	†Untreated for control

 $E_{415}^*$  of 0.2 mg 2-naphthol in 2 ml  $C_2H_5OH-0.02$  ml reagent is

†All rats were approximately the same weight (200-220 g) and had tumours of approximately the same size (1 g).

Analogous results were obtained on repeating all experiments. We have therefore shown that previous glucose administration selectively enhances tumour glucuronidase activity. This has implications for the design of anti-cancer drugs<sup>9,10</sup>; inactive glucuronides of active drugs, for example, the O-glucuronide of p-hydroxyaniline mustard<sup>11</sup>, could be selectively activated in tumour tissue by this procedure. The synthesis of similar conjugates and investigations about its anti-cancer activity are in progress.

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#### Fatigue in frog single muscle fibres

WHEN a whole striated muscle is stimulated repetitively, its contractile force decreases and it is said to be fatigued1-3. Fatigue does not occur in dystrophic muscles, and is characteristic of fast but not slow mammalian and amphibian muscle fibres<sup>4,5</sup>. The complexity of the excitation-contraction process in fast muscle fibres has made it difficult to determine when fatigue sets in. Restoration of twitch tension of fatigued fibres by caffeine<sup>6.7</sup> suggests that fatigue is a failure of calcium release, although the depletion, during prolonged contraction, of necessary energy-rich metabolites8.9 could be responsible for fatigue. Contraction can be uncoupled from calcium release and excitation by hypertonic solutions (refs 10-12 and unpublished results of S. R. Taylor, R. Rudel and J. R. Blinks). We have now found that hypertonic uncoupling prevents fatigue, so that fatigue must be linked to contraction. We have also found that single muscle fibres can recover after being fatigued by prolonged tetanisation.

Single, fast muscle fibres of the semitendinosus muscle of frogs (Rana pipiens) were used, rather than whole muscle, to avoid the rate-limited diffusion and the heterogeneous fibre

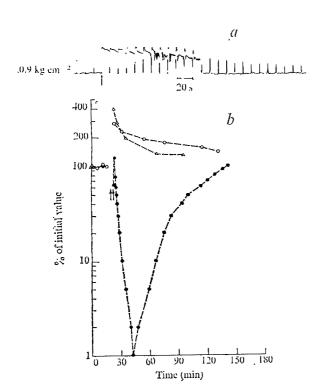


Fig. 1 a, Tension recording from a single muscle fibre tetanically stimulated at 20 Hz. Arrow indicates onset of stimulation. Recovery intervals correspond to 1 s without stimulation. Twitches are shown before and immediately after the tetanus. b, Logarithmic graph of peak tension ( $\bullet$ ), contraction time ( $\cup$ ), and relaxation time ( $\wedge$ ) of twitches from the same experiment as in a. Values plotted are percentages of pre-tetanic levels. The absolute initial values of the parameters are respectively 1.14 kg cm<sup>-2</sup>, 56 ms and 125 ms. The period between arrows indicates the tetanisation period.

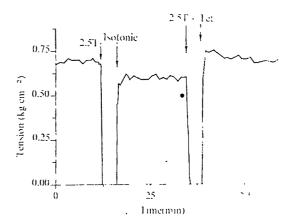


Fig. 2 Twitch tension blockage and recovery from immersion in Ringer made 2.5 times (T) isotonic by addition of Na SO<sub>1</sub>. Arrows indicate immersion period. During the second immersion the fibre was tetanically stimulated at 20 Hz for 120 s.

population in whole muscle<sup>13,14</sup>. A fibre was dissected and mounted in a bath of flowing medium at 15 °C. Tension was recorded by a RCA 5734 transducer tied to one end of the fibre, and was displayed on an oscilloscope screen and a paper chart recorder. Sarcomere length was measured by reading the interline distances of a laser diffraction pattern of the fibre 15, and was adjusted to 2.3 µm. The fibre was stimulated by external platinum electrodes with 0.3 ms condenser discharges. Isometric twitch tension was between 0.7 and 1.2 kg cm 2 and tetanic tension at the beginning of tetanus varied from 1.6 to 2.2 kg cm<sup>-2</sup>.

Figure 1a shows a typical response of a fibre to tetanic stimulation at 20 Hz for 120 s with interruptions of 11 s. During stimulation, tetanic tension decreased to 54% of its initial value in a graded rather than in an all-or-none fashion<sup>16</sup>. In the experiment, the twitch tension that followed the tetanus initially was lower than pre-tetanic control, increased transiently within 10 s and then decreased to close to zero at 10 min (Fig. 1b). It returned to the baseline during a prolonged recovery period of about 100 min (Fig. 1b).

The initial and short-lived increase in twitch tension after a tetanus represents post-tetanic potentiation, which is ascribed to a redistribution of calcium within the sarcoplasmic reticulum<sup>18</sup>. The increase is associated with prolongation of contraction time (time-to-peak tension) and of relaxation time(timefrom-peak tension to baseline, Fig. 1b), also characteristic of post-tetanic potentiation16-18.

In contrast to the prolonged recovery of twitch height after tetanic stimulation in isotonic Ringer (Fig. 1b), recovery of twitch height after immersion in hypertonic Ringer (made 2.5 times (T) isotonic by adding sucrose or Na2SO1) was not affected by tetanic stimulation (Fig. 2). Fibres stimulated in hypertonic Ringer recover their basal twitch tension with a half time of  $53 \pm 15$  s.e. (n = 4) s, compared with a half time of recovery in isotonic Ringer of  $72 \pm 7$  s.e. (n - 3) min. As Fig. 2 shows the rate of recovery of twitch height after tetanic stimulation in hypertonic solution did not differ from the rate after simple immersion. The latter process depends on diffusion of water and hypertonic solute between bath and fibre 13.

These results indicate that the decrement in twitch tension . characteristic of fatigue in single muscle fibres is not due to an intrinsic failure of the calcium release mechanism in excitationcontraction coupling. Contraction is a process that consumes energy<sup>8,9</sup>. Tetanic contraction fatigues the muscle fibre probably by depleting energy stores, which in turn may affect the release of calcium so that twitch tension is reduced.

We thank Drs S. R. Taylor, R. Rüdel and J. R. Blinks for showing us their unpublished records about aequorin luminescence during immersion in hypertonic solutions. J. L. V. is a recipient of a postdoctoral fellowship from the Muscular Dystrophy Associations of America.

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#### Effect of dantrolene sodium on calcium movements in single muscle fibres

Dantrolene sodium or 1-[5-(p-nitrophenyl) furfurylidene amino] hydantoin sodium hydrate (DaNa) is a long-acting drug with skeletal muscle relaxant properties which has recently seemed clinically useful in the treatment of some types of muscle hypertonia<sup>1,2</sup>. DaNa does not affect neuromuscular transmission nor the electrical properties of skeletal muscle<sup>3</sup> and it apparently involves the processes of excitation-contraction coupling<sup>1,3,4</sup>. The mode of action of DaNa has not been studied before or single muscle fibres and here we provide direct evidence that DaNa interferes with the intracellular movements of calcium ions.

Rana temporaria (60 g) kept at 15° C and force fed twice a week with 1-1.5 g chopped beef were used. Single skeletal muscle fibres of the fast twitch type were isolated by microdissection from the dorsal part of the semitendinosus muscle. The fibres diameter 70-150 µm were kept in a standard phosphate Ringer solution<sup>5</sup> at pH 7.2 and they maintained their physiological properties (including electrical threshold) for several hours. When dissected, the muscle fibre was transferred into a small (1.5 ml) Perspex chamber and mounted horizontally. with one of its tendons hooked to the anode of a RCA 5734 mechano-electrical transducer tube. A home-made Perspex four-way tap allowed the thorough replacement of the chamber fluid within about 1 s. A continuous circulation system with appropriate bypasses maintained the temperature of the experimental solutions and of the chamber at 18° C. Electrical pulses of 0.2 ms duration and 1.2 times threshold were delivered

through a pair of bright platinum wires fixed 10mm apart in the bottom of the chamber at right angles to the fibre. DaNa freshly dissolved in phosphate Ringer was applied at a concentration of 10 µM. This produced a mean depression of the twitch force by 58% (eight experiments) within 30 s and an additional 6% decrease during the next 10 min (Fig. 1a). The twitch contraction time and relaxation time were shortened. The action of DaNa was much more rapid for these single muscle fibres than for the whole muscles studied previously. Most of our data were collected within about 6 min of exposure DaNa. The effect of the drug was reversible on return to normal Ringer but a complete recovery of the twitch force took 30-60 min.

When the isometric twitch force was markedly reduced by DaNa, the tetanus force for a 1.5 s supramaximal stimulation at  $100^{-1}$  s was not significantly reduced. The finding of a large reduction of the twitch with little, if any, change in tetanus tension bears out the earlier results on whole muscle<sup>1,4</sup> and indicates that DaNa does not impair the maximum capacity of the muscle fibre to produce force. The recorded reduction of the twitch force by DaNa must therefore be related to an inhibition of some step in the process of mechanical activation, as the membrane spike generation is not impaired3.

A useful way to approach this problem is by recording single fibre contractions to known increases in the external potassium concentration whereby Hodgkin and Horowicz mechanical threshold<sup>6</sup> can be estimated. Figure 1b shows a near maximum contraction of a fresh muscle fibre suddenly exposed to 50 mM

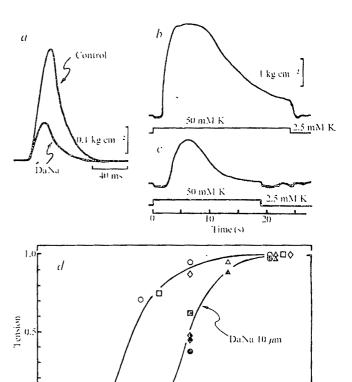


Fig. 1 Effect of DaNa on single muscle fibre. a, Superimposed isometric twitches elicited by supramaximal pulses before and in presence of DaNa; b and c, contractions elicited by a rapid increase in external K from 2.5 to 50 mM (see step), b, before and, c, in the presence of DaNa. The vertical calibration is expressed in kg force output per cm<sup>2</sup> cross sectional area of the muscle fibre. d, Relationship between peak contracture force and the increase of the external K (log scale).  $\bigcirc$ ,  $\bigcirc$ ,  $\triangle$ ,  $\diamondsuit$ , before DaNa;  $\clubsuit$ ,  $\blacksquare$ ,  $\spadesuit$ ,  $, \bullet$ , in presence of 10  $\mu$ M DaNa. Each different symbol corresponds to a different muscle fibre: O diameter 86 μM, maximum tension 3.1 kg cm<sup>-2</sup>; ☐ Maineter 74 μM, maximum tension 3.4 kg cm<sup>-2</sup>; △ A diameter 98 μM, maximum tension 3.7 kg cm<sup>-2</sup>; ◆ ♦ diameter 118 μM, maximum tension 2.5 kg cm<sup>-2</sup>. Temperature of solutions 18° C.

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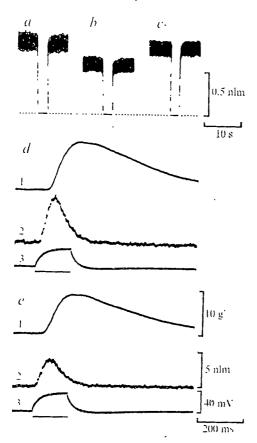


Fig. 2 a-c, Effect of 35 μM DaNa in 0 Ca-1 mM ethyleneglycolbis-(β-aminoethyl ether) N,N'-tetraacetic acid (EGTA) artificial seawater on the resting glow of an isolated barnacle muscle fibre injected with aequorin. The horizontal dotted line represents the 0 level. a, Resting glow after 3 min equilibration in 0 Ca-EGTA; b, decrease of the resting light emission after 30 s in DaNa; c, recovery in 0 Ca-EGTA seawater. Fibre diameter 1.1 mm; mean intracellular resting potential 55 mV. d and e, Result of applying a single electrical stimulation of 150 ms duration to a barnacle muscle fibre injected with aequorin, before, d, and after, e, 6 min exposure to DaNa 35 μM in the external medium E. Trace 1, isometric tension; trace 2, calcium mediated light emission; trace 3, membrane response. Fibre diameter 1.8 mm. Mean intracellular resting potential - 59 mV. Temperature of solutions 21 C.

K. Four min after bathing the fibre in 10 μM DaNa (Fig. 1c) a second exposure to 50 mM K elicited a contraction which was reduced by more than 50%. The latency of this contraction was increased and its rate of rise reduced while the subsequent spontaneous relaxation during the maintained K exposure was quicker than in Fig. 1b. These effects of DaNa were reversible. The graph of Fig. 1, d shows similar data for different concentrations of K in four different muscle fibres. The peak force of each K contraction is expressed with respect to the maximum force recorded in the same fibre during exposure to 117 mM K. These maximum contractions were not significantly affected by 10 μM DaNa in the present single fibre experiments and we think the reduction reported by others' must have been related to their use of whole muscle preparations. The effect of lower K concentrations, however, was markedly reduced and the curve was shifted to the right by a factor of about 1.7. The mean threshold for K contractions was approximately 20 mM in untreated fibres and 35 mM K after 1 min in 10 µM DaNa.

The observation that DaNa reduces both submaximal potassium contractions and twitch force without significantly affecting the maximum potassium contractions or the tetanus force could be explained by an inhibitory effect on some intracellular step in excitation-contraction coupling. To analyse this possibility, single muscle fibres from the barnacle *Balanus nubilus*<sup>7</sup> were injected with aequorin, the calcium-sensitive

bioluminescent proteins. Several thousands of the jellyfisl Aeguorea forskalea were collected at Friday Harbor Labora tories during summer, 1973, and the extracted aequorin wapurified in Brussels, using standard techniques". Lach barnacle muscle fibre of 1.0-2.0 mm diameter was cannulated with  $\epsilon$ Pyrex tube of 200 µm diameter and mounted vertically in a light-tight black Perspex chamber. The calcium-mediated light output was recorded on a Tektronix oscilloscope with ar EMI 9635 photomultiplier tube. When the injected muscle fibre was placed in front of the photomultiplier, the rate of light emission related to the intracellular ionised calcium (resting glow) showed a rapid decrease during the first 30 min and then stabilised at a rather steady level as the [Ca], is very low in the resting state and the rate of consumption of the injected aequorin is small. In these conditions, the replacement of the normal seawater by DaNa seawater was followed by a decrease of the resting glow to a lower level which depends on the drug concentration.

This reduction of the intracellular Ca concentration by DaNa could result either from a reduced resting Ca<sup>2</sup> influx, or from an increased resting Ca<sup>2</sup> efflux or from a direct action involving the intracellular Ca stores. To distinguish these possibilities, the aequorin-injected muscle fibres were tested in 0 Ca 1 mM EGTA artificial seawater, and DaNa 35 µM still reversibly reduced the resting glow by about 20% (Fig. 2a c) which suggests that the DaNa effect is not dependent on any reduction in resting Ca<sup>2</sup> influx. The second alternative could also be excluded in other experiments in which the leakage of <sup>45</sup>Ca into external seawater was estimated in barnacle muscle fibres loaded by an intracellular injection of 0.1–0.2 µl <sup>45</sup>Ca in Tris buffer at pH 7.2 (ref. 10). The resting calcium efflux was actually decreased by 35 µM DaNa which implies that the reduced intracellular calcium cannot result from an increased loss.

Therefore it would seem that DaNa acts primarily on the intracellular Ca storage sites to reduce the level of free sarcoplasmic calcium. This third alternative is indeed supported by studies of the calcium transient recorded during single contractions of barnacle fibres injected with aequorin. The fibre was stimulated at intervals of at least 20 s through an intracellular silver wire with a constant current pulse of 150 ms while the membrane potential was recorded with another intraceilular platinum electrode connected to a high input impedance amplifier8. Although the actual membrane depolarisations thus elicited were identical before and after DaNa (third trace in Fig. 2d and e), the mechanical force (first trace) produced by the stimulated muscle fibre was reduced by 20% after DaNa 35 µM while the transient increase in aequorin luminescence (second trace) decreased by about 30% in this example. These results were regularly recorded in the six different barnacle muscle fibres studied, each one being tested with various combinations of stimulus durations and intensities.

In conclusion, DaNa depresses the mechanical force output of single muscle fibres by an inhibitory effect involving the calcium movements at intracellular storage sites, both at rest and during electromechanical activation.

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#### Simultaneity, occlusion and the sodium pump

GARRAHAN and Garay<sup>1,2</sup> have shown that for the sodium pump of the human red blood cell, the half-saturation concentration for transport of a particular cation from one face of the membrane is not affected by the type or concentration of cation at the opposite face. This finding is consistent with previous observations on the squid axon3 and the sheep red blood cell4. The simplest explanation of these results, according to Garrahan and Garay<sup>2</sup>, is that the pump binding sites are at equilibrium with cation and that transport occurs only when cations are bound simultaneously at both faces of the membrane. Their view is that previously proposed "sequential" models<sup>5</sup> must be rejected and replaced by models in which cation binding sites are present simultaneously on both sides of the membrane. In addition, they show that the transport data are sufficient to reject a model (Fig. 1c) if the pump exists for a substantial time in a conformation in which bound cations are occluded. Yet there is biochemical evidence6 that, at least at 0° C, the pump does nonetheless exist in an occluded state for an appreciable time. Here we show that this apparent paradox is resolved by the tetrameric internal transfer model<sup>7</sup> of Fig. 1d.

The simplest 'simultaneous' models are shown schematically in Fig. 1; the applicable steady state kinetic schemes and their solutions are shown in Fig. 2 (for models a and d of Fig. 1) and in Fig. 3 (for models b and c). We first consider the model of Fig. 1a, Here, a binding site which is sodium-selective in one conformation moves physically across the membrane and becomes potassium-selective in the other conformation8. In the kinetic solution (see Fig. 2), the halfsaturation concentrations for transport, being simply the constants  $K_S$  and  $K_P$ , are independent of the trans cation as required by the experimental data<sup>1-4</sup>. This model makes no provision, however, for an occluded form and in Fig. 1b we have generalised to allow for this possibility. But in doing so we see from the kinetic solution (Fig. 3) that the half-saturation concentrations are no longer  $K_S$  and  $K_P$ but are variables which depend on the trans concentration of cation. Only if the concentration of the occluded form is a negligible fraction of the doubly-bound open forms will the experimental transport observations be satisfied; this conclusion is valid even for a more general model with a succession of occluded forms.

Figure 1c and d show two types of internal transfer model<sup>9,10</sup>. A basic feature of such models is the presence of occluded forms. During each conformation change, transportable substrate is moved only part of the way across the membrane, since the binding sites themselves move only. between a bathing solution and an internal cavity. Within this internal cavity, substrate can move from one binding site to the complementary site. During a second conformation change, substrate can then move out of the internal cavity and into the opposite bathing solution. Garrahan and Garay<sup>2</sup> have shown that the dimer model (Fig. 1c) yields the kinetic solution of Fig. 3, so that once again the concentration of the

occluded form would have to be insignificant. Finally, for the tetrameric internal transfer model (Fig. 1d), the simple kinetic scheme and solution of Fig. 2 are appropriate, because the steps labelled 'transport' now represent the release of substrate which entered the internal cavity during the preceding conformation change.

In summary, of the four 'simultaneous' models of Fig. 1, only the tetrameric internal transfer model both accounts for the experimental transport observations1-4 and allows for the existence of an occluded form during an appreciable fraction of the transport cycle. Thus, to distinguish between these models, one need only determine whether or not the fully-bound forms of the pump exist to an appreciable extent in occluded states.

What appears to be an occluded form of the pump from guinea pig kidney has been detected by Post et al.6. From their data it is possible to obtain a rough estimate of the

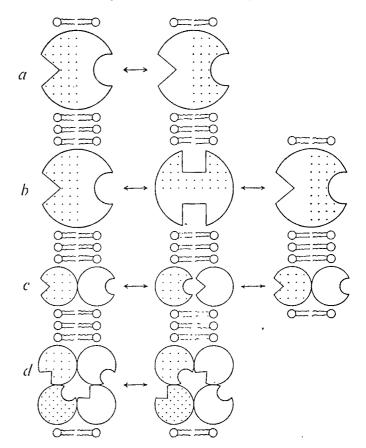


Fig. 1 Simple 'simultaneous' models for active transport. For each model, transport of substrates (not shown) occurs as a result of transitions between the depicted conformations of the transport protein. The substrate binding sites are the sharp, round, and square indentations; the sharp sites are specific for one substrate, the rounded for the second substrate, while the square sites are of undefined specificity. Notice the changes in specificity of a given site from one conformation to another. Only those portions of the transport proteins immediately adjacent to these binding sites are shown; stippling is used to distinguish these regions during the conformation changes. For example, although the two conformations in model a are functionally equivalent, the stippling indicates that they are not physically equivalent, but that the sites have moved completely across the membrane and switched their specificities during the conformation change. Notice that models b-d possess sites in occluded locations, while c and d are in addition internal transfer models<sup>0.10</sup>. Two postulates made for all models are that the binding sites are in equilibrium with the substrates and that conformation changes occur only when all of these sites are fully occupied. (Although each site may require the binding of more than one substrate particle to be completely occupied, the analysis is simpler and the essential points are unaffected by taking the case of only one particle per site.) The drawings are of course wholly schematic, describing the formal basis of the transport events but not the detailed structural features of the transport proteins.

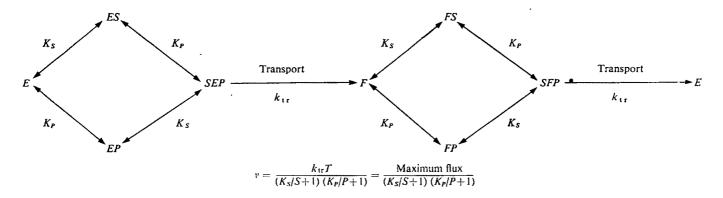


Fig. 2 Kinetic scheme and steady-state solution for the transport models of both Fig. 1a and d. S and P refer to transportable substrates and their concentrations in the left-hand and right-hand bathing solutions, respectively. E and F refer to the two conformations when the outward-facing binding sites are unoccupied, whereas ES, EP, SEP, FS, FP, and SFP refer to these conformations when the outward-facing sites are occupied by S and/or P.  $K_S$  and  $K_P$  are the equilibrium dissociation constants. The rate constant for the transport event is  $k_{tr}$  and T is the total concentration of pump. The unidirectional flux of S in exchange for P, which of course equals the corresponding flux of P in exchange for S, is given by  $\nu$ ; the solution for  $\nu$  is the same as that previously obtained by Garrahan and Garay² for the model of Fig. 1a. Although it is here assumed that each bathing solution contains only a single type of substrate, introduction of competing substrates results in the same expression for  $\nu$  but with the values of  $K_S$  and  $K_P$  effectively increased. Notice that for the model of Fig. 1d all forms of the pump contain in addition two substrate particles in an occluded state.

lifetime of this form at 0° C. Under the conditions of their Fig. 10, they consider the rate of rephosphorylation of the pump enzyme to be largely determined by the rate of disappearance of this occluded form. The maximum measured rate was obtained with high levels of ATP and had a half-time of about 3 s, from which (on dividing by 1n2) the average lifetime of the occluded form would be some 4–5 s. But perhaps rephosphorylation occurs only after the occluded form disappears, so that this is an overestimate. Post *et al.*<sup>11</sup> found, however, that the phosphorylation reaction by itself is complete in less than 1 s at 0° C. Thus it seems that the occluded form exists for some 3–5 s at 0° C.

To determine for what fraction of the total time the occluded form exists, it is necessary to know the average time for a complete pump cycle. This information can be obtained from the experiments of Kanazawa et al.<sup>12</sup> with the rabbit brain enzyme at 0° C. Under the conditions of their Fig. 3 which are comparable to those of Post et al.<sup>6</sup> the K-stimulated (Na+Mg)-ATPase activity was about 0.11  $\mu$ mol P<sub>i</sub> h<sup>-1</sup> per mg protein. The maximum incorporation of <sup>32</sup>P from <sup>32</sup>P-ATP (Table 2 of ref. 12) was  $1.35 \times 10^{-4}$   $\mu$ mol per mg protein, yielding an average cycle time of 4.4 s. This time may be marginally underestimated due to sub-maximal incorporation of <sup>32</sup>P. Using ouabain binding and the ionic binding of ATP to estimate pump concentrations<sup>13</sup> one obtains turnover

numbers at 37° C averaging some 9,000 min<sup>-1</sup> whereas from Fig. 3 and Table 2 of ref. 12 one calculates a value of 11,000 min<sup>-1</sup>; a correction factor of 1.2 might, therefore, be necessary, giving an average cycle time of up to 5.5 s. Overall, taking the range 3–5 s for the lifetime of the occluded form and 4–5.5 s for the cycle time, it seems that at 0° C the sodium pump spends a very substantial fraction of its time in an occluded form. (This time is presumably spent waiting for the rare thermal event of having sufficient energy to overcome the activation barrier between conformations. The time required for the conformation change itself may be rather short (see, for example, the half time<sup>14</sup> of 14 ms for a photon-induced conformation change of rhodopsin in frog retina at 0° C).)

It would thus seem that the first three models of Fig. 1 must be rejected. We are hesitant, however, to adopt this conclusion unequivocally until either the transport observations are confirmed at 0° C or the occluded form is demonstrated at body temperature, or both these findings are confirmed at some intermediate temperature. Nonetheless, there is some indication that the effect at 0° C of ATP binding to a low affinity site and accelerating the release of an occluded form<sup>6</sup> occurs also at 37° C. At this temperature, binding of ATP to a low affinity site accelerates both the (Na+K)-ATPase activity of the bovine brain enzyme<sup>15</sup> and the K:K exchange across human red blood cell membranes<sup>16</sup>.

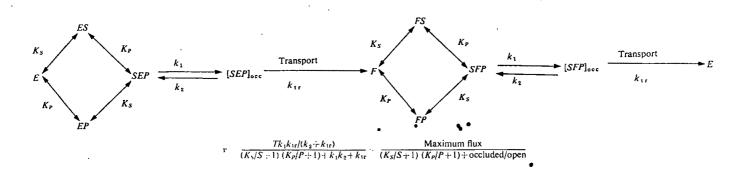


Fig. 3 Kinetic scheme and steady-state solution for the transport models of both Fig. 1b and Fig. 1c. Symbols and comments are as in Fig. 2, with the following additions:  $k_1$  and  $k_2$  are rate constants for reversible non-transporting transitions between open and occluded conformations;  $[SEP]_{occ}$  and  $[SFP]_{occ}$  refer to the occluded doubly bound forms of E and F, respectively; and the ratio 'occluded/open' =  $[SEP]_{occ}/SEP = [SFP]_{occ}/SFP$ . The kinetic solution is that of Garrahan and Garay².

It is encouraging that powerful rejection criteria can be devised on the basis of simultaneity and occlusion. With such criteria one can narrow down the number of possible models for active transport and so reach a clearer understanding of the molecular mechanisms involved.

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# Lateral interaction between neural channels sensitive to velocity in the human visual system

MacKay1 has made use of the phenomenon of simultaneous contrast to provide evidence for the existence in the human visual system of neural channels that are sensitive to the density of visual texture. Using the same phenomenon, we have provided comparable evidence for the existence of lateral interaction between channels sensitive to velocity. That such channels do exist is suggested by the preliminary psychophysical observations of Pantle and Sekuler2, who demonstrated a luminance threshold elevation for moving contours that is limited to a range of values around the velocity of the adapting contour.

A 10×6 matrix of dots was generated on the CRT display of a PDP-12 laboratory computer. The ten symbols in each row were programmed to move at a constant velocity in a west-east direction, giving the impression of a continuous stream of dots drifting across a window. A sharp discontinuity (or border) was generated by having the upper set of three rows move at a slower speed than the lower set. Viewed from a distance of 0.8 m the rows and columns of the matrix subtended visual angles of 3° and 1.88° respectively.

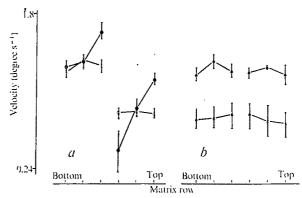


Fig. 1 Perceived velocity of each of the six rows in the matrix of dots, with each point representing the average of three observations. The vertical bars delineate the range of values spanned by the two extreme observations in each case.  $a, \bullet$ , The perceived velocity profile obtained when velocities of 0.30 degree s<sup>-1</sup> and 0.6 degree s<sup>-1</sup> were assigned, respectively, to the upper and lower rows in the matrix. The profile adds confirmation to the illusion reported by several observers; O, with the fast and slow moving rows presented to separate eyes (the left and right respectively) simultaneous contrast does not occur. b, Profiles obtained in control trials in which all six rows were assigned the same velocity, being in the one case 0.6 degree s<sup>-1</sup> ( $\triangle$ ) and in the other, 0.3 degree s<sup>-1</sup>( $\triangle$ ).

With velocities fixed at 0.3 degree s<sup>-1</sup> and 0.6 degree s<sup>-1</sup>, for the upper and lower rows respectively, the authors and several colleagues have experienced an illusion that is consistent with lateral interaction effects. More specifically, the difference in velocity of the rows adjacent to the discontinuity appeared enhanced when compared with the corresponding difference between more outlying rows (for example, the top compared with the bottom row). Perhaps counter-intuitively, the regular columnar organisation seemed to assist in the appreciation of the illusion; the apparent differences in velocity being translated into noticeable deviations from this regularity.

In order to determine more precisely the perceived velocity 'profile' of the matrix, a probe, or comparison row of eight dots was added to one side of the display. Two parameters of this row were made variable: (i) its vertical position could be programmed to coincide with any one of the six rows in the matrix, and (ii) its velocity could be altered by repositioning a smooth lever. By matching the comparison row to each of the six rows of the matrix, an estimate of the perceived velocity profile could be obtained.

One of the authors (P.W.) overcame the initial difficulties that were experienced in trying to both observe the illusion and match the velocity of a selected row and provided the profile illustrated in Fig. 1a. For comparison, the same procedure was followed whilst the dots in all six rows moved with the same velocity, being in the one case 0.6 degree  $\ensuremath{s^{-1}}$  and in the other 0.3 degree s<sup>-1</sup> (Fig. 1b). A comparison of the different profiles highlights the simultaneous contrast character of the illusion. Furthermore, incorporated in Fig. 1a are the results from a condition involving the presentation of the 'fast' and 'slow' moving rows to separate eyes (the left and right respectively). That the illusion does not occur in this condition is consistent with MacKay's results with texture density, and suggests that the lateral inhibitory processes responsible for the illusion reside in the monocular systems, before the point of binocular fusion.

To summarise, the demonstration suggests that the human visual system incorporates channels that are sensitive to velocity and that these channels interact to enhance any discontinuities in this parameter. That there are such channels in the visual systems of cat and monkey is suggested by the discovery of single cells that respond differentially to the speed of a moving stimulus. Such units have appeared in the superior colliculus,

suprasylvian gyrus, and visual area 19 of cat, and in the primary visual cortex of monkey3. The present data may be taken to suggest that with velocity, as with orientation<sup>4</sup>, lateral inhibition effects will be observed at this single cell level.

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# Electrophysiology of mammalian spinal cord in vitro

Mammlian spinal cord has often been used in electrophysiological studies where the experiments were carried out exclusively on the spinal cord in situ1. An obvious disadvantage of such experiments is that the concentrations of ions or drugs studied cannot be controlled precisely in the extracellular medium. To overcome that disadvantage, we have developed an isolated spinal cord preparation of the newborn rat.

Wistar rats aged from 0-7 d were used. Under ether anaesthesia, the spinal cord was isolated, hemisected sagittally and placed in a bath perfused with Krebs solution equilibrated with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. The temperature in the bath was kept at 27±1° C. Changes of the membrane potentials of the spinal motoneurones were followed by recording from the ventral root with an extracellular electrode used in two ways. In the first, the ventral root (L3-L5) was led out through a vaseline-sealed slit in the lateral wall of the bath into a paraffin compartment where the root was placed on a Ag-AgCl electrode, which in turn was connected to a preamplifier and an oscilloscope or pen recorder. The bath was grounded through a reference calomel electrode. In the second method, the ventral root was introduced into a glass suction electrode (Fig. 1a). The portion of the ventral root between the spinal cord surface and the orifice of the suction electrode was covered by a mixture of liquid paraffin and vaseline contained in the glass

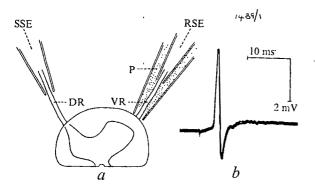


Fig. 1 a, Experimental arrangement. The recording suction electrode (RSE) was connected through a Ag-AgCl electrode to a preamplifier. SS E, stimulating suction electrode; VR, ventral root; DR, dorsal root; P, liquid paraffin. b, Mono- and polysynaptic reflexes induced by a supramaximal volley in L5 dorsal root and recorded from the corresponding ventral root. Positivity upwards.

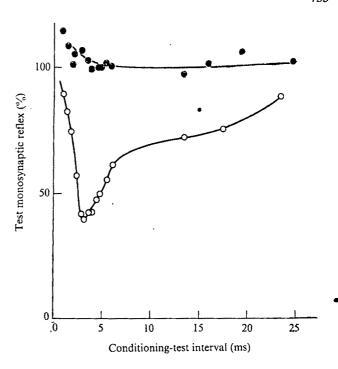


Fig. 2 Postsynaptic inhibition and the effect of Cl deficiency. Test reflex response was induced by a supramaximal volley in L5 dorsal root and was recorded from the corresponding ventral root. Single supramaximal conditioning volley was delivered to L4 dorsal root. Graph shows the amplitudes of the test monosynaptic reflexes expressed as percentages of the unconditioned control and plotted against the conditioning-test intervals, O, in normal Krebs solution; •, in Cl-deficient medium, where 9/10 of Cl ions were replaced by equimolar methylsulphate and methanesulphonate.

cuff. The cuff surrounded the inner suction electrode and was slightly pressed against the surface of the spinal cord. A quite stable d.c. recording was possible in these conditions.

Stimulation of the dorsal root (L3-L5) induced an early sharp spike followed by the later asynchronous waves recorded from the corresponding ventral root (Fig. 1b). The early spike was interpreted as the monosynaptic reflex because its wave form resembled that of monosynaptic reflex observed in the in situ experiments<sup>2</sup> and because the delay time of the spike (about 3 ms in the experiment shown in Fig. 1b) could be explained for the most part by the conduction time in the afferent and efferent pathways3. The preparation was quite stable and a good reflex response could be recorded for at least several hours.

Repetitive stimulation of the dorsal root at 0.3-1 Hz caused a marked decline of the sizes of mono- and polysynaptic reflexes. After tetanic stimulation, a slight increase in the amplitude of monosynaptic reflex (about 130% of the control) was observed. In the experiment illustrated in Fig. 2, L5 dorsal root was stimulated at 0.1 Hz and the reflex response was recorded from the corresponding ventral root. Conditioning stimulation of the adjacent dorsal root (L4), which by itself produced only very slight potential changes in the L5 ventral root, exerted during the following period of several milliseconds a remarkable inhibitory effect on the monosynaptic reflex. Judging from the fact that this inhibition displayed a relatively fast time course and was readily blocked by strychnine (5×10<sup>-6</sup> g ml<sup>-1</sup>), we conclude that it is the postsynaptic inhibition1. When the concentration of chloride ions in the external medium was reduced to one-tenth of the normal, the postsynaptic inhibition was completely abolished (Fig. 2). This strongly indicates that the permeability increase to chloride ions is of predominant importance in this type of inhibition (compare with refs 1, 4 and 5).

The isolated spinal cord preparation described here seems to provide many possibilities for electrophysiological, pharmacological and developmental studies of the spinal cord.

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## Excitatory action of hypothalamic substance P on spinal motoneurones of newborn rats

It is generally believed that the primary afferent fibres constituting the spinal dorsal roots, form excitatory synapses with the neurones in the spinal cord and the brain stem<sup>1</sup>. Several substances have been considered as the neurotransmitters of the primary afferent neurones<sup>1-5</sup>. We have found that an undecapeptide, substance P, which has been isolated from bovine hypothalamus<sup>6,7</sup>, exists in the dorsal root of bovine spinal nerve in an amount 10-30 times larger than that in the ventral root<sup>8,9</sup>, and that this peptide exerts a potent excitatory action on the spinal motoneurones of the frog<sup>8,10</sup>.

These findings led us to postulate a hypothesis that hypothalamic.substance P is a neurotransmitter of the primary afferent neurones and, to explore this hypothesis further, we examined the effect of hypothalamic substance P on the neurones of the mammalian spinal cord. The isolated spinal cord preparation of the newborn rat is suitable for this purpose because substances can be applied by adding to the perfusion

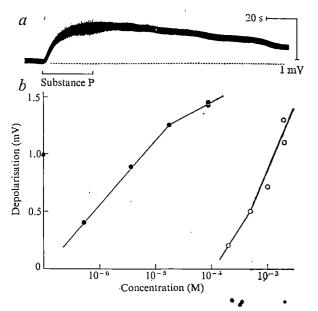


Fig. 1 a, The potential recorded from the L4 ventral root of an isolated spinal cord of a newborn rat. Hypothalamic substance P (9×10<sup>-5</sup> M) was bath-applied during the period indicated. Depolarisation is upwards; dotted line indicates the control level. b, Dose-response curves of hypothalamic substance P ( ) and L-glutamate ( ), obtained in another preparation. Ordinate, amplitude of the induced depolarisation recorded from L4 ventral root; abscissa, molar concentration of the applied drugs on a logarithmic scale.

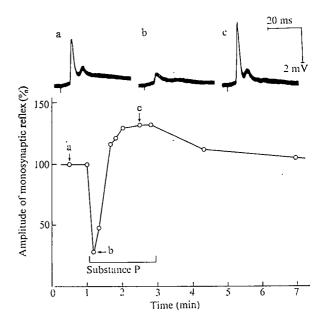


Fig. 2 Effect of hypothalamic substance P  $(3 \times 10^{-5} \text{ M})$  on the spinal reflex. L4 dorsal root was stimulated supramaximally at 0.1 Hz and the reflex responses were recorded from the corresponding ventral root. Graph shows the amplitude of the monosynaptic reflex plotted over time. Substance P was bath-applied during the period indicated in the graph. Upper oscillographic traces show sample records at the times indicated by arrows in the lower graph.

fluid in controlled concentrations, which enables us to make an exact estimate of the relative potencies. The method of recording the electrical activities generated in the motoneurones was the same as described in the accompanying paper<sup>11</sup>.

When Krebs solution in the bath was replaced by the solution containing synthetic hypothalamic substance P, a depolarisation accompanied by the spike discharges of spinal motoneurones was recorded from the ventral root (Fig. 1a). After the bath was washed out with Krebs solution, the recovery took place with a slow time course of a few minutes. L-Glutamic acid of relatively high concentrations induced similar depolarisation and spike discharges but the time course of the response was faster than that of substance P. In Fig. 1b, the relationship between log molar concentration and the induced depolarisation was plotted for both hypothalamic substance P and L-glutamate. From the comparison of the dose-response curves at the level of 1 mV potential change, the potency of the depolarising action of substance P was estimated to be about 200 times higher than that of L-glutamate which is regarded as a leading candidate for the excitatory transmitter in the spinal cord<sup>2</sup>.

To find out whether hypothalamic substance P causes the depolarisation by directly acting on the motoneurones or by a trans-synaptic mechanism, the effect of the peptide was re-examined after the intraspinal synaptic transmission was blocked in a low-Ca and high-Mg solution. When the preparation was soaked in a modified Krebs solution containing 0.4 mM Ca and 7 mM Mg, the ventral root reflex evoked by dorsal root stimulation was completely abolished. At this stage the depolarisation induced by substance P was as large as that in normal Krebs solution, suggesting that the peptide has a direct excitatory action on the spinal motoneurones10.

Figure 2 shows the effect of hypothalamic substance P on the spinal reflex. First, the peptide induced a transient inhibition followed by a potentiation of the monosynaptic reflex. The first inhibitory effect of substance P may be due to the activation of the GABA-operated inhibitory mechanism because this inhibition was almost completely abolished after the treatment of the preparation with picrotoxin (10<sup>-5</sup> g ml<sup>-1</sup>). The second facilitatory effect, on the other hand, can be explained by the depolarisation of the motoneurones.

Thus the present results give further support to the hypothesis that hypothalamic substance P may serve as an excitatory transmitter of the primary afferent neurones in the spinal cord.

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## A new type of hereditary persistence of foetal haemoglobin: is a diffusible factor regulating y-chain synthesis?

PERSISTENCE of foetal haemoglobin synthesis into adult life produces a condition known as hereditary persistence of foetal haemoglobin (HPFH). Different types of HPFH have been described but the precise molecular mechanism(s) responsible for them has not been clarified. Several genetic hypotheses have been proposed, of which the most widely accepted is that of a deletion in the δβ complex; this would agree with the fact that both the gene(s) for HPFH (so far described) and the β-structural locus behave as alleles. We have studied a new type of HPFH in a negro family, the gene for which does not behave as an allele of the δβ complex, and have therefore proposed a genetic interpretation for this condition.

The index case was found during the course of a systematic re-examination of patients previously diagnosed on clinical grounds as cases of sickle-cell anaemia. The pedigree and the haematological data of the family are shown in Fig. 1 and Table 1. The pedigree shows that a βs, a βthal and a gene for the persistence of foetal haemoglobin at approximately 5% level, are segregating in this family. While  $\beta^s$  and  $\beta^{that}$  behave as usual (they are found separately in I<sub>1</sub> and I<sub>2</sub>, together in II<sub>1</sub>,

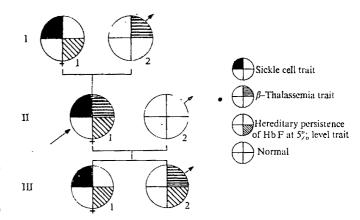


Fig. 1 Pedigree of the family AMR. The arrow indicates the proposita.

and segregate again in III, and III, the mode of transmission. of the gene for HPFH is very strange. In fact, II<sub>1</sub> who has the responsible gene in a single dose (her father has normal levels of foetal haemoglobin (HbF)), transmits it together with the βs (both genes received from I<sub>1</sub>) and the β<sup>thal</sup>. The phenotype of I<sub>1</sub> also seems to be anomalous: an inherited form of persistence of HbF coexists with a normal HbA/HbS ratio (see Table 1), although in every form of this condition so far described, there has always been total or partial differential depression of the β gene in the cis form, with respect to the HPFH mutation<sup>1</sup>.

This is the first clear example in which a gene causing persistence of HbF does not behave as an allele of the δβ complex, as indicated by the fact that both \( \beta^s \) and HPFH sites, together in the gamete transmitted from I<sub>1</sub> to II<sub>1</sub>, were present in the gamete transmitted from II<sub>1</sub> to III<sub>1</sub>. Furthermore, only the HPFH site was transmitted from II1 to III2: in this case the locus for the persistence of HbF and that of  $\beta^{that}$  previously separated, must have travelled together in the gamete which II<sub>1</sub> has transmitted to III<sub>2</sub>. This individual carries both the genes for \( \beta^{that} \) and for HPFH, as indicated by the high levels of HbA2 and HbF. The latter finding cannot be explained by the variability of HbF in βthat heterozygotes, because it has been shown that HbF levels show intrafamilial segregation in β<sup>thal</sup> (ref. 9); and the grandfather of III<sub>2</sub>, who transmits the high-A<sub>2</sub> β<sup>thal</sup> gene, only shows 0.6% HbF. Moreover, the gene causing persistence of HbF does not alter the HbA/HbS ratio. Each of these two findings suggests that the site of mutation of the persistence of HbF in this family is located in a gene different from the classical HPFH gene.

The haematological, biochemical and genetic data of this family can be explained by the following assumptions: (1) The abnormality results from persistent γ-chain synthesis from one of the  $\gamma$ -chain loci in  $I_1$  which passes on to  $III_1$  through  $II_1$ . (2) The coexistence of both genes for β<sup>that</sup> and HPFH in III<sub>2</sub>

				Т	able 1 Ha	ematolo	gical data	of famil	y AMR				
I <sub>1</sub> I <sub>2</sub> II <sub>1</sub> II <sub>2</sub> III <sub>1</sub> III <sub>2</sub>	Age (yr) 48 53 28 30 9	Hb (g%) 11.8 12.8 8.6 14.4 13.8 10.1	RBC (10 <sup>8</sup> mm <sup>-3</sup> ) 4.55 6.16 4.08 4.50 5.15 5.21	Het (%) 40 47 32 41 44 37	MCV (μm³) 88 76 78 91 85 71	MCH (pg) 26 21 21 32 27 19	MCHC (%) 29.5 27 27 35 34 27	HbF (%) 5.3 0.6 16.0 0.2 5.2 6.8	HbA <sub>2</sub> (%) 2.8 5.0 4.0 2.4 2.9 5.3	HbS (%) 42 80 42	Osmotic resistance + +++ +++ Normal ++++	106 118 88 103	Intracellular distribution of HbF  Heterogeneous Heterogeneous Heterogeneous Heterogeneous

+. Slightly increased; ++, increased; +++, very increased. Haematological indexes and osmotic fragility were obtained by standard methods<sup>2,3</sup>. Red blood cells (RBC) counts were performed in an Automatic Cell Counter (TOA Microcell Counter, Mod. CC-1002). Haemoglobin electrophoresis was performed according to the method of Smithies. An electrophoretic technique was also used for the quantitation of HbA and HbS. HbA2 was quantitated by the chromatography method of Berninis, and HbF by the alkali denaturation method of Betker. The amount of serum iron was determined by the sulphonated bathophenanthroline method8.

is the consequence of a cross-over between the  $\gamma$  and  $\beta$  loci, a highly improbable event because of the linkage between the two loci<sup>10,11</sup>. In fact, this would be the first example of a recombination between HPFH and βs sites. (3) This hypothetical mutation, producing persistence of HbF, does not affect the expression of the β-structural gene in the cis form.

It is evident that many assumptions have to be made to fit all the data within the classical scheme. On the other hand, all the findings are to be expected assuming that the mutation under discussion is located in a locus which does not affect γ-chain expression by an intrachromosomal interaction. It is tempting to suggest that there exists a locus which affects the  $\gamma$ -chain expression via a diffusible substance. This would be the first example in mammals of a regulatory mechanism affecting the synthesis of a polypeptide chain by a diffusible substance.

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# Sulphydryl groups as a new molecular probe at the $\alpha_1$ $\beta_1$ interface in haemoglobin using Fourier transform infrared spectroscopy

THE haemoglobin molecule consists of a pair of subunits, each of which is made up of two different haem-bearing polypeptide chains,  $\alpha$  and  $\beta$ . The contacts between the two  $\alpha\beta$  subunit pairs are not covalent and constitute the  $\alpha_1\beta_2$  (and  $\alpha_2\beta_1$ ) interface. Symmetrical dissociation of the haemoglobin molecule occurs relatively easily at this interface. Large changes in the  $\alpha_1\beta_2$ contacts occur² as the molecule switches from the T to the R conformation during ligand binding3.

The association of the  $\alpha_1\beta_1$  (and  $\alpha_2\beta_2$ ) contact is much stronger. Although this contact is formed largely by nonpolar van der Waals interactions, asymmetrical dissociation along the  $\alpha_1\beta_1$  interface is more difficult to achieve and little change occurs here during ligand binding<sup>2</sup>. Reactions of the sulphydryl (SH) groups of cysteinyl residues α-104 (G11) and especially  $\beta$ -112 (G14), located at the  $\alpha_1\beta_1$  interface, are very useful in dissociating the αβ subunit4.5.

These residues may also be involved in intracellular haemoglobin denaturation occurring in thalassaemia and unstable haemoglobinopathies as well as in interaction of denatured haemoglobin with cell membranes. In spite of its importance to haemoglobin structure and stability, the  $\alpha_1\beta_1$  interface and its hidden SH groups have remained inaccessible to available

We report here the first observation of infrared absorption bands due to SH groups in a native protein. The observed infra-

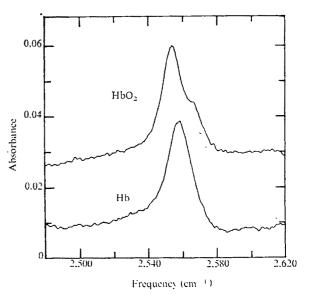


Fig. 1 Infrared spectra of 17 mM (haem) human oxyhaemoglobin and deoxyhaemoglobin against 16 mM bovine carboxyhaemoglobin and deoxyhaemoglobin, respectively. Single beam spectra of human haemoglobin samples were taken in a cell with a 0.2 mm optical path and CaF<sub>2</sub> windows using a Digilab Model FTS-14D interferometer equipped with a liquid nitrogen-cooled InSb detector. Four sets of 64 coherently added interferograms were independently subjected to Fourier transformation and were, in turn, co-added to yield a digital single beam spectrum at 2 cm resolution. This was initially compared with a single spectrum of air to produce an absorbance spectrum of the sample. A similarly obtained digital absorbance spectrum of H<sub>2</sub>O was then subtracted from the sample spectrum. The water content of the samples was determined from a near infrared absorption band of H<sub>2</sub>O at 1.92 μm. Bovine haemoglobin spectra were treated similarly and subtracted from the human haemoglobin spectra. Haemoglobin solutions were buffered to pH 7.1 bisTris and were 0.1 M in chloride.

red absorption bands are the result of S-H stretching vibrations of cysteine residues at the  $\alpha_1\beta_1$  interface ( $\alpha$ -104 (G-11) and β-112 (G-14)) of native human haemoglobin A. Centre frequencies and absorption intensities of these SH groups provide structural information about haemoglobin in aqueous solution that has been available in no other way.

The absorption bands due to cysteine SH groups at the  $\alpha$ -104 (G-11) and β-112 (G-14) positions of human oxyhaemoglobin and deoxyhaemoglobin, respectively, are shown in Fig. 1. These were measured with Fourier transform infrared (FTIR) interferometry (with a Digilab Model FTS-14 Fourier transform spectrometer), and are corrected for absorptions due to water and to protein by subtraction of the appropriate spectrum of bovine haemoglobin, which contains cysteine only at the  $\beta$ -93 position. The latter residue is predicted to have only a very weak, broad absorption band that is not observed in our conditions. The use of bovine haemoglobin as reference assures that only cysteine SH groups at the  $\alpha$ -104 and  $\beta$ -112 positions in human haemoglobin will be observed. Assignment (J.O.A., G.H.B., and P.A.B., unpublished) of these absorption bands to S-H stretching vibrations is based on: first, comparison with small molecule thiols; second, loss of the absorption band at alkaline pH; and third, observation of a pair of bands with a similar absorption envelope near 1,855 cm<sup>-1</sup> in D<sub>2</sub>O but not in  $H_2O$ , that have a calculated isotopic frequency ratio  $(v^*/v)$ equal to 0.7268, which is virtually identical to the corresponding values calculated from the data<sup>8</sup> reported for methanethiol vapour (0.7267) and liquid (0.7270).

The spectrum of human oxyhaemoglobin SH groups (Fig. 1) clearly contains two overlapping bands of differing intensities. Quantitative band shape analysis shows that the apparently more symmetrical deoxyhaemoglobin SH spectrum is also composed of two overlapping bands. Horse and pig haemoglobins contain cysteine residues at only the α-104 and β-93 positions7. Their oxyhaemoglobin spectra, when compared with

Table 1 Infrared intensities of sulphhyd	ryl absorptions
	ε <sub>mM</sub> (area)* (mM <sup>-1</sup> cm <sup>-2</sup> )
0.1 M ethanethiol in CCl <sub>4</sub> 0.1 M ethanethiol in H <sub>2</sub> O 5.36 M <i>n</i> -propanethiol in acetone	0.070 0.210 0.62†
HbA: 8.5 mM $\alpha_1\beta_1$ dimer $\alpha$ -104 Cys (G-11) $\beta$ -112 Cys (G-14)	2.7 0.80

<sup>\*</sup>  $\varepsilon_{mM}$  (area) =  $[1/(cl)][\log_{10}(I_0/I)dv$ † From ref. 10.

bovine haemoglobin (β-93 cys), show only a single symmetrical absorption band of similar frequency and intensity to the lower frequency, more intense, SH absorption band of human haemoglobin. A band corresponding to the lower intensity SH shoulder from human oxyhaemoglobin is not present in spectra from horse or pig. We therefore assign the band at 2,553.8 cm<sup>-1</sup> to v(SH) of the  $\alpha$ -104 cysteine in human oxyhaemoglobin, and the shoulder due to a band at 2567 cm<sup>-1</sup> to v(SH) of the  $\beta$ -112 cysteine. Corresponding bands are observed at 2,557.0 cm<sup>-1</sup> and 2,564 cm<sup>-1</sup> in human deoxyhaemoglobin. These frequencies are sensitive to the state of ligation of haemoglobin, and differ in the order HbCO < HbO<sub>2</sub> < HbCN < Hb<sup>+</sup>  $\ll$  Hb, for the α-104 v(SH) (J.O.A., G.H.B., and P.A.B., unpublished).

The reasons for the sensitivity of these sulphydryl groups to small changes in molecular structure may be understood by consideration of the observed absorption intensities (Table 1). Monomeric thiols absorb very weakly in the infrared unless they are polarised by molecular interactions such as H bonding9. Thus, H bonding by solvent water produces about three times the integrated intensity observed in CCl<sub>4</sub>, and acetone produces a further threefold increase in intensity. The integrated intensity of propanethiol in acetone<sup>10</sup> is somewhat less that due to the β-112 cysteine SH, but less than one fourth of that due to the α-104 cysteine SH. We therefore propose that both of the SH groups at the  $\alpha_1\beta_1$  interface in haemoglobin must be strongly H bonded, probably to a peptide carbonyl of the fourth residue back in the G helix. Such an H bond seems consistent with Perutz' suggestion that certain serine residues may form similar H bonds<sup>11</sup>. This possibility was tested by construction of molecular models of the G helices for horse oxyhaemoglobin (Labquip Co., Caversham, UK) from coordinates attributed to

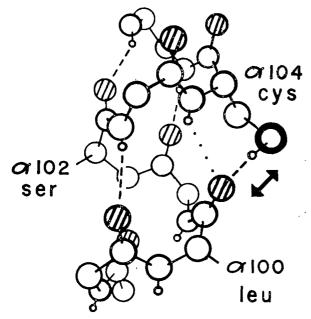


Fig. 2 Drawing of the a chain G helix of horse oxyhaemoglobin from α-98 (G-5) to α-105 (G-12), prepared from a photograph of a molecular model. The amino acid side chains are not included except for that of a-104 (G-11) cysteine. The oxygen atoms are shaded and the sulphur atom is in bold face.

Perutz. The G helix of horse  $\beta$  chain contains no cysteine, and the amide NH groups all seem to be fairly well aligned with corresponding amide carbonyl groups four residues back. The same observation is made for the G helix of the horse a chain except for the amide carbonyl of  $\alpha\text{-}100$  (G-7 leu), which seems to stick out sharply from the helix (Fig. 2). This carbonyl is in a very favourable position to form a strong H bond with the SH group of the α-104 (G-11) cysteine, which would satisfy the infrared intensity requirements. We predict that a somewhat lesser distortion of the human  $\beta$  chain G helix may be found, which would permit an amide carbonyl at β-108 or β-109 to form a somewhat weaker H bond with the  $\beta$ -112 cysteine SH, which may be shared with an amide NH group of β-112 or  $\beta$ -113, respectively. A weaker H bonding of the  $\beta$ -112 cysteine SH group would agree with the weaker infrared absorption intensity and higher frequency of the band assigned to this group (Fig. 3). Polarisation and rotational fixation of the  $\beta$ -112 SH by H bonding may partially account for the greater reactivity with iodoacetate of the  $\beta\text{-}112$  as compared to the  $\beta\text{-}93$ sulphydryl in isolated human haemoglobin \beta chains observed by Neer12.

$$\alpha$$
-Chain:  $\alpha$ -104(G-11)CysS—H.... O=C  $\alpha$ -100(G-7)Leu β-Chain:  $\beta$ -112(G-14)CysS—H.... O=C  $\{\beta$ -108(G-10)Asn or  $\beta$ -112(G-14)  $\{\beta\}$ -112(G-14)  $\{\beta\}$ -113(G-15)  $\{\beta\}$ -113(G-15)  $\{\beta\}$ -113(G-15)

Fig. 3 Proposed H bonding for the  $\alpha$ -104 (G-11) and  $\beta$ -112 (G-14) cysteine SH groups in human haemoglobin.

This model can account qualitatively for the observed sensitivity of v(SH) to differences in the state of haem ligation. Change in quaternary structure upon ligation results in a relative displacement of atoms at the  $\alpha_1\beta_1$  contact by about 1 Å (ref. 2). The contribution of altered van der Waals contacts to v(SH), however, should be small unless there is an alteration of polar group association with the sulphydryls. Alternatively, changes in tertiary structure may be responsible. Slight bending of the G helix would be geometrically amplified to change the S-H .. O=C hydrogen bond distance (Fig. 2, arrow), or a slight rotation of the α-100 peptide carbonyl could cause a change in the strength of the hydrogen bond association. Although these possibilities remain to be tested, Fourier transform infrared spectroscopy of haemoglobin sulphydryl groups clearly provides a sensitive molecular probe for the observation of structural changes in the  $\alpha_1\beta_1$  interface region resulting from tertiary

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or quaternary conformational changes of the protein.

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#### NMR evidence for tertiary structure base pair in E. coli tRNA involving S<sup>4</sup>U<sub>2</sub>

In previous nuclear magnetic resonance (NMR) studies of tRNA we observed anomalously low field resonances (14.5-15.0 p.p.m. downfield from the usual standard 2,2-dimethylsilapentane-5-sulphonate (DSS)) in the spectra of many, but not all, Escherichia coli tRNA1-3 but yeast tRNA we examined never exhibited such resonances1,2. The fact that approximately 60% of the E. coli tRNA are class I and have a 4-thiouridine (S4U) residue at position 8 (refs 4 and 5), whereas yeast tRNA do not, raised the possibility that the anomalous low field resonances in E. coli tRNA might be due to ring NH protons of S'U8 in a tertiary structure base pair.

To test this possibility, NMR spectra of S'U and U mononucleosides were compared in dimethylsulphoxide (DMSO) and we found that the ring NH proton of U was at 11.5 p.p.m. (ref. 6) whereas the corresponding resonance of S'U was at 12.6 p.p.m. (K.L.W. and D.R.K., unpublished). Assuming comparable hydrogen bond strengths, these model system studies suggested that the ring NH proton of an S'U-A base pair would be located further downfield than the corresponding resonance of an AU base pair. As resonances from AU base pairs can be located as far downfield as 14.5 p.p.m. (refs 1-3), these observations prompted us to re-examine the assignment of the anomalous low field resonances in E. coli tRNA. For this study we chose to work with unfractionated tRNA to determine whether or not base pairing with S'U<sub>8</sub> is a common feature in E. coli tRNA. As we shall show, a tertiary structure base pair involving S'U<sub>8</sub> is responsible for the anomalous low field resonances in the NMR spectra of E. coli tRNA.

Unfractionated E. coli tRNA was obtained from Plenum Scientific (Lot no. 73042). Three different procedures were used to convert S'U to U, including IO<sub>3</sub> oxidation (K.L.W. and D.R.K., unpublished), photo-oxidation7,8 and cyanogen bromide treatment<sup>9,10</sup>. The conversion of the S<sup>4</sup>U to U was monitored by following the disappearance of the S'U absorption spectrum and in each case at least 90% of the S'U residues were converted. NMR samples were prepared free of chemical reagents and then dialysed against solutions containing appropriate amounts of Mg2+, cacodylate and NaCl. A typical sample contained ~25 mg ml-1 of tRNA, 0.1 M NaCl, 10 mM Mg and 10 mM cacodylate buffer, pH 7. NMR spectra were obtained with a Varian HR 300 spectrometer operated in the field sweep mode. All spectra were extensively (1-2 h) signal averaged using a Nicolet 1020A signal averager. The low field NMR spectrum of the untreated E. coli tRNA is shown in Fig. 1 along with the spectra of the chemically modified samples. All spectra were taken at 25° C.

As expected on the basis of our earlier examination of several pure E. coli tRNA species, unfractionated E. coli tRNA exhibits a broad, rather weak resonance located around 14.8 p.p.m. with only ~0.025 times the intensity of the resonances located between 11.5 and 14.5 p.p.m. Resonances in the 14.5-11.5 p.p.m. region have previously been assigned to the ring NH protons of AU or GC Watson-Crick base pairs (one resonance per base pair)1-3

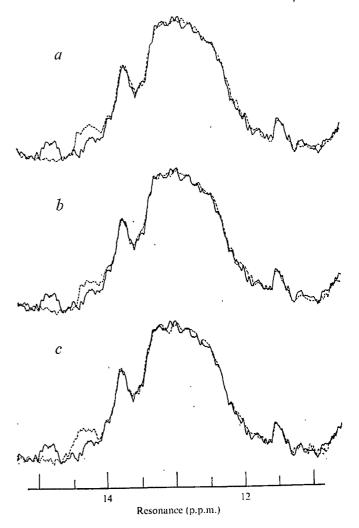


Fig. 1 A comparison of the 300 MHz proton NMR spectra of unfractionated E. coli tRNA before (——) and after (——) chemical conversion of S<sup>4</sup>U to U. Spectra were obtained at 25° C and the positions of the resonances are in p.p.m. downfield from DSS. Additional experimental details are given in the text. Three differential chemical procedures -) and after were used, including a, photo-oxidation; b, cyanogen bromide treatment; and c,  $\mathrm{IO_3}^-$  oxidation. As these comcyanogen parisons demonstrate, the only major changes in the spectra occur in the 14.8 and 14.3 p.p.m. regions of the spectra. All other features in the spectrum remain virtually unchanged.

and integration of these spectra indicates there are an average of 20±2 resonances per tRNA<sup>1-3</sup>. Thus, the intensity in the 14.8 p.p.m. region corresponds approximately to ~0.5 proton (base pair) per molecule. This number is consistent with our attribution of the 14.8 p.p.m. resonance to S'U as about 60% of the E. coli tRNA are class I and have an S'U residue at position 8 (refs 4 and 5).

To prove that S'U residues are responsible for the anomalous low field resonances three different chemical procedures were used to convert S'U to U. The effect of each of these on the low field NMR spectrum is shown in Fig. 1. In each case the S⁴U→U conversion causes the loss of the 14.8 p.p.m. resonance and the appearance of a new resonance at around 14.3 p.p.m. On the basis of these observations we conclude that the anomalous 14.8 p.p.m. resonance in E. coli tRNA is due to a tertiary structure base pair in which S'U8 is hydrogen bonded with another base located elsewhere in the molecule. Consideration of the low field position of the resonance (before and after conversion to U), and the relative strengths of in-plane ring current shifts from G and A suggest that an adenine is the other base paired with S'U<sub>8</sub>.

The following observations suggest that A at position 14 is the one paired with S'U8. It has been demonstrated that

S'Us can be photochemically crosslinked with C13 in several different E. coli species without significantly affecting the biological activity11,12. As expected, we also observed that photocrosslinking  $S^4U_8$  and  $C_{13}$  resulted in loss of the 14.8 p.p.m. resonance (K.L.W., and D.R.K., unpublished). Furthermore, conversion of S'U to U has little effect on the biological activity of the E. coli tRNA (refs 8 and 10). Taken together, these results indicate that in a biochemically active conformation the arms of the cloverleaf are folded with S'Us located close to C13 and therefore also close to the A residue which is always present at position 14 (refs 4 and 5). On this basis, the 14.8 p.p.m. resonance is assigned to a tertiary structure base pair between S'U8 and A<sub>14</sub>. The formation of such a base pair was proposed some time ago by Levitt's on essentially the same grounds, but this is the first experimental evidence that S'U8 is actually involved in a tertiary structure base pair. Recent X-ray diffraction data show that A14 and U8 are close together in yeast tRNA Phe crystals, but the resolution is not adequate to show whether they are actually hydrogen bonded<sup>13,14</sup>. As a new resonance appears in the E. coli tRNA spectrum at 14.3 p.p.m. when  $S^4U_8$  is converted to U<sub>8</sub>, there should be a similar resonance from a tertiary structure A-U<sub>8</sub> base pair in yeast tRNA. Re-examination of previously published spectra indicates that this is probably true<sup>1-3</sup>.

Based on Leonard's structural studies of the S'U-C photodimer<sup>15,16</sup> and his model for the arrangement of S'U<sub>8</sub> with respect to C<sub>13</sub> in tRNA (ref. 17), and previous NMR studies of yeast tRNAPhe which indicates that A14 is stacked on GC<sub>13</sub> (ref. 18) we have to conclude that the S'U<sub>8</sub>-A<sub>14</sub> base pair is not Watson-Crick. If a few additional tertiary structure base pairs can be identified, then it may be possible to determine the complete folding of tRNA molecules in solution, particularly when this information is combined with the results of rare earth metal binding studies18,20. Because the A-S'U(U) tertiary structure base pair is not part of a regular double helix it should be very sensitive to solvent conditions (salt, Mg2+, temperature) which are known to stabilise or destabilise the folding of tRNA. A detailed re-examination of the NMR spectra of some pure species and further work on chemical modifications is currently under investigation.

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## Possible role of DNA synthesis in formation of sister chromatid exchanges

As there is now evidence that an eukaryote chromosome is composed of a single molecule of double-stranded DNA1,2, sister chromatid exchanges (SCEs) detectable in mitotic chromosomes of higher organisms can be regarded as a kind of recombination between two homologous DNA duplexes. The SCE formation is readily enhanced by exogenous agents such as ultraviolet light and chemical mutagens and seems to be closely related to post-replicational repair process in mammalian cells3. Precise knowledge about the time and sites of the SCE formation might help greatly to understand the mechanism underlying this phenomenon and its biological significance. Previous attempts3 to pinpoint cellular phases in which this recombination process is initiated have been hampered by the usage of 3H-thymidine which, although indispensable for the demarcation of sister chromatids, would cause strand scissions at any cellular phases.

A chromatid consisting of DNA substituted with 5-bromodeoxyuridine (BUdR) can be clearly distinguished from one containing no BUdR using fluorescence staining techniques3-5, and visible light causes strand breaks in BUdR-substituted DNA regions<sup>6,7</sup>. Taking advantage of these facts I here present circumstantial evidence that the site of the SCE formation is strictly limited to DNA portions which are undergoing or just have completed replication.

Chinese hamster cells growing exponentially, D-63, were labelled with BUdR at various concentrations for 5 h in the dark, washed and incubated further for 21 h in fresh medium containing 10 µg ml<sup>-1</sup> thymidine, and then fixed for chromosome preparation following 1 h pretreatment of cells with 0.05 µg ml<sup>-1</sup> colcemid. The slides were stained with 0.125 mg ml-1 acridine orange3. Most metaphase cells were in the second post-labelling mitosis (generation time; 16th). Visible light illumination to cells labelled with BUdR was carried out at room temperature during the second post-labelling cell cycle at various time intervals from collection. The light source was a 20 W blue fluorescent light bulb (National) which emitted no ultraviolet light. Culture dishes containing 2 mm deep medium were placed at a distance of 5 cm from the bulb. The energy flux of the incident light in these conditions has not been determined. DNA synthetic activity of cells was determined autoradiographically in BUdR-substituted duplicate cultures by labelling cells with 3H-thymidine during the period of visible illumination.

Effects of the concentration of BUdR and visible illumination on the frequency of SCEs are shown in Fig. 1 and Table 1. In non-illuminated contröls, the frequency of SCEs remained unchanged at BUdR concentrations 0.25-2.5 µg ml-1. At higher concentrations it increased with increasing concentration. Visible illumination readily provoked SCEs in cells labelled with 1.0 µg ml<sup>-1</sup> BUdR. In the following experiments,



Fig. 1 Chinese hamster chromosomes stained with acridine orange. The cell was labelled with BUdR (1.0 μg ml<sup>-1</sup>) for 5 h and illuminated with visible light in the second post-labelling cell cycle for 20 min at 8 h before collection (× 1,680).

cells were labelled exclusively with 1.0  $\mu g$  ml  $^{-1}$  BUdR and received 20 min visible illumination.

Figure 2 shows the time relationship of DNA synthetic activity of cells and the incidence of SCEs in No. 1 chromosomes. It is clear that the formation of SCEs was enhanced only when visible illumination was carried out in the S phase. As for the failure to induce SCEs in G2, there may be a possibility that the G2 chromatin might be resistant to the photolysis by visible light because of its condensed state and protection by chromosomal proteins. I thought this unlikely, however, as chromosomal aberrations were readily induced in cells illuminated with visible light at G2. Without illumination, the frequency of chromatid breaks and achromatic lesions was about 0.03 per cell whereas in cells illuminated at 2 h before collection, it was 0.28 per cell.

The parallel between the increase in SCE frequency and in DNA synthetic activity was also found in the X chromosome (Fig. 3a). The X of Chinese hamster is a biarmed chromosome, the short arm replicates in the earlier part of S while the long arm in the later part of S. The X as a whole starts DNA synthesis about 2 h behind the other chromosome complements such as No. 1 chromosomes (Figs 2 and 3a). The increase in the SCE frequency over the control level was detected only when this particular chromosome was under replication. SCEs induced by visible illumination were observed exclusively

Table 1 Frequencies of SCEs in BUdR-labelled cells with or without visible illumination during the second post-labelling cell cycle

BUdR (µg ml <sup>-1</sup> )	Visible illumination* (min)	SCEs per No. 1 chromosome†
0.25		0.44
0.5		0.45
1.0		0.44
2.5		0.42
5.0		0.58
10.0	_	0.85
20.0	<del></del>	1.62
40.0		2.61
1.0	0	0.44
1.0	5	1.58
1.0	. 10	2.12
1.0	20	3.22

<sup>\*</sup> Illumination was carried out 8 h before fixation of cultures.
† 100 chromosomes each were scored in non-illuminated controls
and 50 chromosomes each in visible illuminated cells.

in the short arm in the earlier part of S whereas in the long arm in the later part of S. Figure 3b shows the ratio of the long arm to the whole X with respects to SCE frequency and DNA synthetic activity. The time when the curves for SCE ratio (Fig. 3b, A and B) crosses the control line is not appreciably different from the time when the ratio for DNA synthetic activity cross the average line; that is, when the majority of DNA-synthesising sites moved from the short arm to the long arm. This seems to indicate that strand breaks responsible for the initiation of recombination process might be those induced in DNA regions which are replicating or have just completed replication. The majority of breaks caused by visible light in BUdR-substituted strands may be repaired without strand exchanges between chromatids7. It is uncertain whether the concurrence of the SCE formation with DNA replication is due to a direct involvement of replication forks in the recombination process or due merely to geometrical constraint in terms of the possibility of the interaction of two broken ends; separation of duplicated DNA strands and their subsequent binding with chromosomal proteins might reduce greatly such a probability.

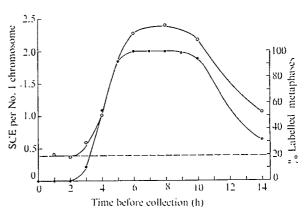


Fig. 2 The formation of SCEs in No. 1 chromosomes in relation to DNA synthetic activity of cells.  $\bullet$ , DNA synthetic activity is expressed as the % labelled metaphases. BUdR-substituted cells were labelled with \*H-thymidine (0.6  $\mu$ Ci ml $^{-1}$ , specific activity 12.1 Ci mol $^{-1}$ ) for 20 min during the second cell cycle while they were being illuminated with visible light. O, The SCE frequency was scored in cultures untreated with \*H-thymidine. ---, Non-illuminated control.

The labelling procedure used here was such that only one of four strands in a metaphase chromosome contained BUdR. As the target of the photolysis by visible light is BUdR-substituted DNA portions, the results obtained in this work may seem to imply that a single break in a BUdR-substituted parental strand is sufficient to initiate a process of the SCE formation. The following alternatives cannot be precluded, however. First, a small amount of BUdR, which is undetectable by the present technique, would be incorporated into other three strands probably at the time of repair of spontaneous DNA lesions<sup>8</sup>, thereby providing two breaks upon visible illumination to initiate the recombination process. Second, visible illumination might cause strand breaks also in DNA unsubstituted with BUdR, though far less efficiently than in BUdR-substituted DNA6, and the SCE formation is initiated by two breaks each made in sister chromatids. As to the latter possibility, it was confirmed that at least DNA lesions detectable as chromosomal aberrations were not caused by visible illumination in cells untreated with BUdR: The number of breaks in cells illuminated with visible light 6 h before collection was found to be 0.06 per cell. This value was not significantly different from that obtained in non-illuminated controls (0.02 chromotid breaks per cell).

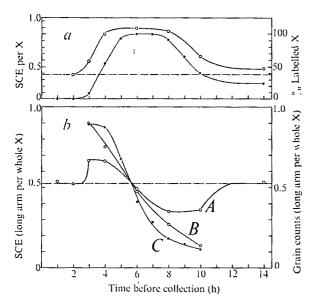


Fig. 3 The formation of SCEs and DNA synthetic activity in the X chromosome examined in the same samples as those used in Fig. 2a, time relationship of the enhancement of the SCE formation by visible light (O) and DNA synthetic activity (O) of the X chromosome. b, Ratios for the SCE frequency and DNA synthetic activity in the long arm of the X to those in the whole X. The SCE ratio in non-illuminated control was 0.524, whereas the average ratio for DNA synthetic activity was 0.526. The latter value was obtained by dividing the sum of the number of silver grains scored on the long arm in each sample by that on the whole X. Both ratios are indicated as a single broken line. Curve A shows the SCE ratio determined based on the net frequency of SCEs and curve B that based on the fraction of SCEs solely due to visible light illumination. Curve C indicates the ratio for DNA synthetic activity assessed by grain count.

The simplest and most attractive models for recombination presume two strand breaks as an initial step which is then followed by a heteroduplex formation by two broken ends thus derived9-11. A speculation that a discontinuously replicating DNA piece at the replication fork might provide a pairing mate to a broken end in a BUdR-substituted strand seems to be attractive and awaits more detailed studies in this respect.

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## Localisation of 5S ribosomal RNA genes on human chromosome 1

We have used in situ hybridisation to locate a major 5S RNA locus near the end of the long arm of chromosome 1 of man. This information represents a step towards the understanding of the synthesis, assembly and regulation of ribosomes. The 5S RNA molecule is associated with the larger ribosomal particle in both higher and lower organisms and is required for ribosome function. Ribosomal RNA-18S and 28S-is synthesised at the nucleolar organiser regions on the chromosomes of the D and G groups1. Genes coding for 5S RNA have been located in Drosophila2, Xenopus3 and several other organisms4, and our data now confirm preliminary reports5.6 that placed the 5S genes on chromosome 1 of man. We also suggest a mechanism for positioning the 5S genes near nucleoli at interphase.

Hybridisation experiments with human DNA on nitrocellulose filters have shown that DNA from the largest chromosomes has more 5S RNA genes than that from chromosomes in the other three size classes7. We have used the recently reported method for localising 5S genes more precisely by RNA:DNA annealing to metaphase figures, using high specific activity 5S RNA labelled with 125I. The 5S RNA was isolated from polysomes of HeLa cells and purified by preparative gel electrophoresis. After iodination8 the RNA was repurified on another column (DEAE cellulose), dialysed extensively against water and lyophilised. This 5S RNA (specific activity about  $2 \times 10^8$  d.p.m.  $\mu g^{-1}$ ) was reconstituted in  $4 \times$  SSC (0.15 M NaCl, 0.015 M sodium citrate) at pH 6.0 at a concentration of 2 µg ml<sup>-1</sup>. The nucleotide sequence of this HeLa 5S RNA has been determined and it was at least 90% pure 5S RNA.

Slides were prepared from cultured leukocytes taken from the peripheral blood of two individuals. We used standard methods involving methanol-acetic acid fixation. Just before hybridisation the chromosomal DNA on slides was denatured in 0.2 N HCl for 20 min. at room temperature, chilled immediately in 0° C 2×SSC, dehydrated in ethanol and air dried. The reconstituted 5S RNA solution was diluted with an equal volume of formamide and used for annealing experiments with chromosome preparations. Background radioactivity and nonspecific binding are reduced considerably if 1-4 mg of commercial Esherichia coli tRNA or yeast 4S and 5S RNA is added to 1 ml of hybridisation mixture. Other details of the procedure have been described8. Autoradiographs were prepared using a 1:1 (w/w) dilution of Kodak NTB with distilled water, exposed for 2 months, developed by standard methods and stained with Giemsa in Sorenson's buffer at pH 7.0.



Fig. 1 Autoradiographs of human chromosomes of the A group hybridised in situ with <sup>125</sup>I-5S RNA. The horizontal lines indicate sets of A group chromosomes from six different cells. The diagrammatic chromosomes agreed on by the Paris conference10 are on the left. (The major chromosome section numbers are indicated but the sub-numbers for individual bands have been deleted because of the low magnification here.)
The 5S genes are placed near q41 or q42 on chromosome 1, which correspond to the first proximal band and interband in 1 q4 indicated by the arrow. The 'noise level' radioactivity on chromosome 2 is randomly distributed. A minor 5S gene site may be on chromosome 3 but the data are inconclusive.

In almost every cell examined, chromosome 1 was labelled at a site near the end of the long arm (q) with an average of 2.8 silver grains per locus. In Fig. 1 the chromosomes in the A group from six cells are compared with the standardised chromosomes agreed at the Paris chromosome conference<sup>10</sup>. Measurements of chromosome 1 that exhibit clear banding at 1q12 place the 5S genes on the long arm at about 1q41. The location of this 5S gene site has been confirmed using 125I-5S RNA to label the chromosomes from two fibroblast cultures, which were heterozygous for different translocations<sup>11</sup>. With one aberration, t(1;13) (q32;q34), the 5S locus on chromosome 1 was translocated to chromosome 13. In the second aberration, t(1;4) (q44;q25), the 5S genes were not translocated to chromosome 4. The data are all consistent in placing the major 5S RNA locus on the long arm of chromosome 1 at or near q41 and within the segment q32 to q44 as bracketed by translocation breakpoints.

The localisation of 5S genes on the remaining chromosomes of the genome is less straightforward. An identified chromosome must be labelled consistently at a particular site, otherwise the data are meaningless. It has not been possible to identify labelled chromosomes with certainty because of variable banding patterns. The information that follows is an attempt to provide tentative assignments for the 5S genes, and provides a qualitative analysis of each chromosome group (A-G) shown in Fig. 2, obtained from the karyotypes of 34 cells at metaphase (20 male and 14 female). (A) Chromosome 1 is the most heavily labelled. Chromosome 2 is not labelled above background, however, 3 may be labelled but at a frequency of silver grains too low to place a site. (B) Chromosomes 4 and 5 are not labelled above noise level binding. (C) One or two pairs of the middle sized chromosomes are labelled. In cells showing distinct C banding, chromosome 9 is usually labelled.

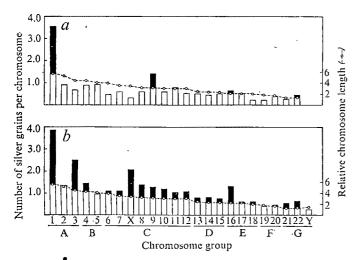


Fig. 2 An analysis of autoradiographs from metaphase chromosomes hybridised with <sup>123</sup>I-5S RNA. Radioactivity and relative chromosome length are plotted according to chromosome number. Chromosomes 1, 2, 3 and 16 are identified with reasonable certainty. The placement of the remaining chromosomes is reliable only within a group according to their measured length. This latter placement is analogous to the older criteria before G and C banding techniques were available. The assignment of the chromosomes in the top set (female 40°C) is more accurate because some chromosomes had prominent C bands, which apply especially to assignments within the C group. a, These chromosomes are from cultured leukocytes of an adult female. The hybridisation with 1 µg <sup>123</sup>I-5S RNA per mt was done at 40°C in 50% formamide in 2×SSC overnight. b, These chromosomes are from cultured leukocytes of an adult male. The hybridisation conditions are the same, except that the slides were incubated at 60°C for one hour before transfer to the optimum temperature at 40°C overnight. A 60°C temperature in 50% formamide will melt the SS DNA. We believe that some non-histone proteins are removed with these conditions because of increased hybridisation efficiency.

When the X chromosome can be identified by banding, it is not labelled. (D) Chromosomes 13, 14 and 15 do not seem to carry 5S genes nor do detected levels of annealing occur at the satellited regions that would indicate contamination from 18S or 28S ribosomal RNA. (E) Chromosome 16 probably has 5S genes. The identification of 16 is reliable because the centromere is located centrally and there is a large C band at q11. Chromosome 17 and 18 are not labelled above background. (F) Neither 19 nor 20 are labelled. (G) Chromosome 22 might have 5S genes but 21 and the Y probably do not, and the Y exhibits less 5S RNA binding per unit length than any other chromosome. Thus besides the definite localisation on 1, tentative assignments indicate that chromosomes 9 and 16 probably have 5S genes. Chromosomes 3 and 22 have above average labelling but remain in question. The overall distribution of the 5S genes is consistent with previous hybridisation studies7.

In interphase nuclei the 5S RNA genes have been observed to cluster around nucleoli in Drosophila12.13, Xenopus3 and the Chinese hamster<sup>14</sup>. (We have verified these results in the Chinese hamster, where the 5S RNA genes are clustered around nucleoli.) With interphase nuclei from mouse Sertoli cells, human leukocytes and human fibroblasts the radioactivity from annealed 5S RNA is associated with nucleoli16. An analysis of interphase nuclei from 100 human fibroblasts shows that 50-70% of the 125I-5S RNA radioactivity is associated with nucleoli. A random distribution would be 20-30%. Centres of radioactivity and lines of 6-10 silver grains are usually over nucleoli and at the edge11. Many of these highly labelled sites should be the 5S genes on chromosome 1. Most nuclei with 3 or 4 nucleoli have label associated with each nucleolus, indicating that more 5S genes are present than on chromosome 1.

The obvious advantage of this nucleolar association would be to synthesise 5S RNA in proximity of nascent particles for effective assembly of 5S RNA into the 60S ribosomal particles. Interphase nuclei seem to possess a mechanism to position certain chromosome segments into a consistent three-dimensional configuration. What is the physical basis for the association of the ribosomal precursor genes?

Certain information about human chromosomes—(a) banding patterns10; (b) the location of C bands and the highly repeated DNA sequence16; (c) the localisation of the ribosomal RNA gene at the secondary constrictions of the D and G group chromosomes 13, 14, 15, 21 and  $22^1$ , and (d) the 5S gene sites described here—shows that the chromosomes with large blocks of highly repeated DNA (C bands) are either nucleolus organiser chromosomes (13, 14, 15, 21 or 22) or chromosomes 1, 9 and 16, reported here to have 5S genes. C-RNA from satellite II anneals to the C band on chromosome 1 at q12 and with the C band on chromosome 16 at q1117. After long exposure, these same in situ preparations gave labelling at the C band on chromosome 9 at q12 and at the nucleolar heterochromatin. Are these highly repeated DNA sequences the recognition sites which position the ribosomal precursor genes?

Ferguson-Smith and Handmaker<sup>18</sup> analysed the position of the nucleolus organiser (NO) chromosomes in relation to the location of other chromosomes in 432 mitotic cells. Chromosome 1 was consistently associated with the NO chromosomes, and direct attachments were illustrated between the C band q12 on chromosome 1 and the nucleolar heterochromatin end of a chromosome in the D or G group (Plate 3A-C of ref. 18). Shaw made a similar observation<sup>19</sup> where the D and G group chromosomes were adjacent to chromosome 1, usually at right angles to the acentric ends of the D or G chromosomes that point toward the centromere of chromosome 1. The evidence suggests that the highly repeated DNA sequences function to orient and position the genes which synthesise the RNA for ribosomes.

Even though the assignments of 5S genes to chromosomes 9 and 16 will require further cytogenetic verification before

their mapping can be accepted, some observations on association should be noted. Using high pH Giemsa staining the large heterochromatic block on chromosome 9 could be recognised at interphase in fibroblasts and Sertoli cells20. This block was often associated with the small nucleoli of fibroblasts and showed a frequency of 84% attachment of Sertoli cell nucleoli. In seven consecutive patients with chronic myelogenous leukaemia, Rowley<sup>21</sup> found that a piece of chromosome 22, an NO chromosome, was translocated to chromosome 9. These two chromosomes must have been closely associated at interphase to produce the translocation.

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# Pseudotype formation between enveloped RNA and DNA viruses

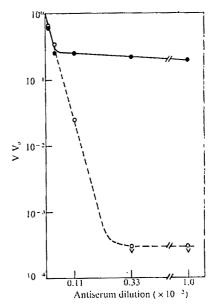
ENVELOPED RNA viruses from different groups participate readily with each other in phenotypic mixing during maturation at the plasma membrane of host cells. These 'pseudotypes' with the envelope antigens of one virus and the genome of the other have been demonstrated for simian virus 5 and vesicular

stomatitis virus (VSV)1, for avian or murine RNA tumour viruses and VSV<sup>2-4</sup>, and for fowl plague virus and VSV<sup>5</sup>. (An exception has been the lack of interaction between Sindbis virus and VSV6.) We have now found virions containing the RNA genomes of VSV in the envelope antigens of a DNA virus, herpes simplex virus (HSV). These interactions between genomes and antigens of unrelated groups may be part of a mechanism for viral pathogenesis and oncogenesis.

Coinfection of cells with HSV and VSV would be expected to result in progeny containing either the HSV or VSV genomes. To differentiate between plaques, the morphology and efficiency of plaque formation by VSV and HSV were examined. HSV was assayed on both human epidermoid number 2 (HEp-2) cells and on Chinese hamster ovary (CHO) cells under liquid or agar overlays, and VSV was assayed on both cell types under agar only. The results of many experiments are summarised here.

The MP strain of HSV7 formed no detectable plaques on CHO cells. (We show below that this restriction was due to the inability of HSV to enter CHO cells.) The Indiana serotype of VSV8, on the other hand, grew well on both types of cell, although the efficiency of plaque formation on HEp-2 cells was only 10% of that on CHO cells.

The MP strain of HSV forms polykaryocytes on HEp-2 cells when the cells are incubated under liquid overlay containing anti-HSV antiserum7. Under agar, HSV on HEp-2 cells appeared as very small, red plaques and small, clear white plaques. HSV plaques were similar whether the agar overlay contained anti-HSV antiserum or not. The white plaques, although similar in size to VSV plaques on HEp-2 cells, were distinguishable by their appearance under the microscope.



Neutralisation by anti-VSV serum of VSV(HSV) pseudotypes formed after mixed infection in the presence of cytosine arabinoside. HEp-2 cells were infected with HSV at a multiplicity of 2 in the presence of 20 µg ml<sup>-1</sup> of cytosine arabinoside and incubated at 37° C for 4.5 h. Then the cells were superinfected with VSV at a multiplicity of 0.01. After each attachment period the cells were washed twice with 10 ml of saline. The cells were covered with MEM containing 2% FCS, 5 µg ml<sup>-1</sup> gentamicin and 20 µg ml<sup>-1</sup> cytosine arabinoside. After a total incubation time of 30 h at 37° C, the extracellular fluid was decanted, cells and cell debris precipitated and the supernatant frozen at -100° C. For neutralisation mixtures the supernatant containing VSV and VSV(HSV) progeny was thawed and mixed with equal volumes of rabbit anti-VSV antiserum<sup>3</sup> at the indicated dilutions. Neutfalisation was for 1 h at 22° C and then the samples were diluted and plaque assayed on either HEp-2 or CHO cells as indicated for Table 1. The log of the percentage of non-neutralised virus  $V/V_0$  is plotted against the antibody concentration.  $\bullet$ , Hep-2;  $\bigcirc$ , CHO.

Table 1 Assay of VSV progeny produced by HEp-2 cells coinfected with VSV and HSV or infected with VSV alone

Virus inoculum VSV + HSV VSV + HSV VSV + HSV VSV + HSV	Anti-VSV* + - +	Anti-HSV† + +	Assay cells HEp-2‡ HEp-2 HEp-2 HEp-2	PFU ml <sup>-1</sup> × 10 <sup>-3</sup> 3.40 1.10 1.80 < 0.01
VSV VSV VSV	- + - +	_ + +	HEp-2 HEp-2 HEp-2 HEp-2	19.00 <0.01 17.00 <0.01
VSV + HSV VSV + HSV	+	<del>-</del>	CHO§ CHO	2.25 < 0.01
VSV VSV	<del>-</del>	<del>-</del>	CHO CHO	5.00 < 0.01

A sample of 4.5 × 10<sup>6</sup> human epidermoid number 2 (HEp-2) cells<sup>17</sup> were infected with the MP strain of herpes simplex virus type I (HSV-1)<sup>7</sup> at a multiplicity of 1 and superinfected 4 h later with vesicular stomatitis virus (VSV) of the Indiana serotype at a multiplicity of 0.4. The infected cells were incubated in Eagle's minimal essential medium (MEM) containing 10% foetal calf serum (FCS) and 5 μg ml<sup>-1</sup> of gentamicin. After 24 h extracellular virus was collected as previously described<sup>18</sup>. A similar sample of HEp-2 cells was mock-infected with HSV then infected with VSV at a multiplicity of 0.4 and collected at the same time as the previous sample. Neutralisation mixtures were made by first diluting antiserum against VSV 1:50 and antiserum against HSV 1:100. Each serum was mixed in equal volumes with each of the two virus preparations which had been diluted previously to yield approximately 10<sup>3</sup>–10<sup>4</sup> PFU ml<sup>-1</sup> on the respective cells. Where both antisera were used against one of the virus preparations, both concentrations of antisera were each identical to the concentration of antiserum when used alone. Neutralisation mixtures were allowed to stand at 22° C for 1 h and then diluted. Unneutralised virus preparations were treated identically except that antisera were absent. Virus in duplicate aliquots of 0.1 or 0.2 ml of the appropriate dilutions were attached to monolayer HEp-2 or Chinese hamster ovary (CHO) cells on 60 mm plastic Petri plates for 40 min at 37° C. The cells were then rinsed with medium and overlaid with MEM containing 2% FCS, 5 μg ml<sup>-1</sup> of gentamicin and 0.9% agar. After incubation at 37° C for 48 h in a 5% CO<sub>2</sub> humidified incubator, the cells were stained with 0.01% neutral red and the plaques counted after another 18 h of incubation.

\* Rabbit anti-VSV antiserum was prepared as described previously<sup>3</sup>. † Anti-HSV serum was human immune serum globulin provided by Dr Michael Oxman and prepared by the Department of Public Health of the Commonwealth of Massachusetts.

‡ HEp-2 cells and their properties have been described before<sup>19</sup>. § CHO cells and their properties have been described<sup>18, 20</sup>.

The efficiency of plaque formation of HSV with a liquid or agar overlay was approximately the same.

When HEp-2 cells were infected with HSV and later superinfected with VSV, results were different depending on the multiplicity of VSV. At high multiplicities (>1) only progeny VSV were detectable in the extracellular medium. These progeny were neutralised by anti-VSV serum whether they were assayed on HEp-2 or CHO cells.

During mixed infection with smaller multiplicities of superinfecting VSV (<1) both VSV and HSV progeny were detected in the extracellular medium. Table 1 shows the results when VSV plaques were recorded. On HEp-2 cells one-third of the VSV progeny produced by the mixed infection was resistant to neutralisation by anti-VSV serum. This resistant VSV population did not form plaques on CHO cells and was neutralisable by anti-HSV serum when assayed in HEp-2 cells. A control sample of progeny from HEp-2 cells infected with only VSV is presented for comparison. Anti-HSV antiserum had no effect on this VSV preparation. These results demonstrate the presence of virions containing VSV genomes covered by HSV envelope antigens which are designated pseudotypes of VSV(HSV).

To ensure that there was no confusion between the VSV and HSV plaques in this experiment, we took advantage of reports that cytosine arabinoside inhibits HSV DNA synthesis without marked inhibition of the synthesis of plasma membrane-associated HSV glycoproteins<sup>9,10</sup>. Because our experiments

were designed to assay only released extracellular virions, the growth of HSV for 28 h under these conditions was determined in the presence of 1–50  $\mu g$  ml $^{-1}$  cytosine arabinoside. The growth of detectable extracellular HSV progeny was inhibited by more than 99.9% at these concentrations of cytosine arabinoside (data not shown). Less than 10° PFU per ml was detected. The same inhibitor at 20  $\mu g$  ml $^{-1}$  had no effects on the synthesis of VSV in HEp-2 cells, nor did it result in marked cytotoxicity of uninfected HEp-2 cells after a 30 h treatment at 37° C.

HEp-2 cells coinfected with HSV and VSV in the presence of cytosine arabinoside should synthesise HSV glycoproteins, but the progeny should contain only those with VSV genomes. To detect VSV(HSV) pseudotypes three different protocols were used for these mixed infections. In each case HEp-2 cells were infected with HSV at a multiplicity of 2 in the presence of 20 μg ml<sup>-1</sup> cytosine arabinoside. These cells were superinfected with VSV at a multiplicity of 0.01, 4.5 and 6.5 h after HSV infection. A third sample was superinfected, 22 h after HSV infection, with VSV at a multiplicity of 50. All three samples were collected 30 h after HSV infection, when the cytopathic effect of VSV was evident.

Of the infectious progeny from these mixed infections 3-20% were resistant to neutralisation by anti-VSV serum. A more detailed characterisation of the sample superinfected with VSV at 4.5 h is shown in Fig. 1 and Table 2. A persistent fraction of 20% was detected on HEp-2 cells after neutralisation with anti-VSV serum (Fig. 1). This persistent fraction was not detected on CHO cells (Fig. 1). Such findings indicate a surface restriction<sup>2,3</sup> for VSV(HSV) on CHO cells and may explain the lack of susceptibility of some Chinese hamster cells to HSV<sup>11,12</sup>.

A control preparation of VSV made in HEp-2 cells in the presence of 20 µg ml<sup>-1</sup> cytosine arabinoside was neutralised by anti-VSV serum (Table 2). In addition, VSV(HSV) from the mixed infection was neutralised specifically by anti-HSV serum (Table 2).

These results confirm the findings that specific alteration of host plasma membranes by HSV occurs although HSV progeny do not use plasma membranes during their maturation<sup>13-15</sup>. Presumably, during budding VSV genomes pick up HSV-specific antigens. Also, VSV(HSV) pseudotypes indicate that enveloped DNA and RNA viruses mix phenotypically. The process appears to be quite efficient because 30% of the VSV population can be pseudotypes. It remains to be shown whether this percentage will be as high when strains other than the macroplaque (MP) strain of HSV are used in mixed infections.

If pseudotype formation occurs between VSV and other herpes-like viruses, the rescue of membrane markers by VSV from human tumour cells<sup>16</sup> might be the result of partial gene

Table 2 Neutralisation of pseudotypes by human immune globulin

Virus	Anti-VSV	Anti-HSV	PFU ml <sup>-1</sup> ×10 <sup>-3</sup>
VSV-CA*	-		1.250
VSV-CA	+		< 0.001
VSV(HSV)†	- Parkers		9.820
VSV(HSV)	+		0.475
VSV(HSV)	_	+	8.620
VSV(HSV)	+	+	< 0.001

Neutralisation mixtures were made with equal volumes of virus and anti-VSV and/or anti-HSV sera. Details of neutralisation and plaque assay on HEp-2 cells were identical to those described for Table 1.

\*VSV-CA was prepared in  $5 \times 10^6$  HEp-2 cells by first treating the cells with cytosine arabinoside at 20 µg ml<sup>-1</sup> in MEM for 6 h at 37° C and then infecting with VSV at a multiplicity of 0.1. The inhibitor was maintained in the medium. After incubation for 19 h at 37° C VSV was collected from the extracellular fluids.

†This preparation of virus was identical to the preparation shown in Fig. 1. Compared with Fig. 1, the reduction in titre of the percentage of PFU resistant to neutralisation by anti-VSV serum probably due to the disruption of HSV envelopes resulting from repeated freezing and thawing of the same virus preparation.

expression of herpes-like viruses as well as RNA tumour viruses. Also, although it is premature to conclude anything about the effect of pseudotype formation on viral oncogenesis, possible interactions of herpesviruses with RNA viruses at the level of host membranes may result in the rescue of defective herpesviruses by other enveloped viruses, and in the rescue of defective RNA tumour viruses by herpesvirus-coded surface

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# Effect of dexamethasone on herpes simplex virus type 2 infection in vitro

MORRHENN et al.1 reported that when polyoma virus-infected cultures of mouse embryo fibroblasts and 3T3 cells were treated with physiological doses of corticosteroids, plaque formation was enhanced tenfold. Because the plaques appeared earlier and were larger in the presence of steroids, they suggested that the rate of production of virus was also increased. It has also been reported that glucocorticoids influence in vitro infections with murine leukaemia virus and mouse mammary tumour virus<sup>2,3</sup>. Since there is epidemiological evidence that herpes simplex type 2 (HSV-2) can be oncogenic in man4 and evidence that inactivated HSV can produce malignant transformation in vitro<sup>5</sup>, we studied the effect of corticosteroids on HSV-2 infection in mouse embryo fibroblasts and 3T3 cells, the two cell

types in which effects had been noted with polyoma virus. We report here that dexamethasone has different effects on HSV-2 infection in vitro depending on both the type of cell tested and the characteristics of the viral strain used.

The MS strain of HSV-2 was obtained from Dr L. W. Catalano and grown in our laboratory in Vero cell cultures in RPMI 1640 medium with 5% feotal bovine serum. Two substrains, one highly syncytia-forming (HSV-2syn) and the other predominantly non-syncytia-forming (HSV-2non-syn), were obtained by plaque purification from the uncloned virus which produced both syncytial and non-syncytial plaques on Vero

Virus pools were grown in Vero cells and titrated by plaque assay with a methylcellulose overlay as before8. 3T3 cells were obtained from Dr George Todaro and Swiss mouse embryo fibroblasts (SMF) were obtained from Microbiological Associates. All experiments were performed with monolayer cultures in 25-cm<sup>2</sup> Flacon plastic flasks.

Dexamethasone (10<sup>-5</sup>–10<sup>-7</sup> M) and cortisol (10<sup>-6</sup> M Sigma) were prepared in absolute ethanol and dissolved to appropriate concentrations in medium or in the methylcellulose overlay. Media for control cultures contained ethanol in amounts equal. to those in the steroid-containing media.

Monolayers of 3T3 cells were preincubated for 24h in glucocorticoid-containing medium, infected with HSV-2 syn and fed with medium containing glucocorticoid, and the total amount of virus produced 24 h after infection was measured by plaque assay in Vero cell cultures6. A tenfold or greater increase in yield of virus was found in several experiments (Table 1).

Table 1 Effect of glucocorticoids on production and plaquing efficiency of HSV-2syn on 3T3 cells

		Viral yield	
Glucocorticoid			No. of plaques*
		(PFU per flask)	
Dexamethasone	10⁻⁵ M	$1.8 \times 10^{\circ}$	82
	10 −6 M	$4 \times 10^{7}$	75
	10 -7 M	$3 \times 10^7$	60
Cortisol	10 <sup>−6</sup> M	$1 \times 10^7$	83
Control		5×10 <sup>5</sup>	16

Flasks containing 3×106 cells were infected with 108 PFU of HSV-2syn. The 24 h viral yield was measured after two cycles of freezing and thawing of cells and supernatant, followed by centrifugation at 1,000 r.p.m. for 10 min to remove cell debris, and then by plaque assay on Vero monolayers

Input 200 PFU as measured on Vero monolayers. Figures are averages of those for two replicate flasks.

To test whether the 3T3 monolayers had become more susceptible to HSV-2syn infection under the influence of the steroids, we studied the plaquing efficiency of the virus with a methylcellulose overlay containing steroids. Repeated experiments showed a twofold to fivefold increase in the number of plaques in the cultures treated with 10<sup>-5</sup>–10<sup>-7</sup> M dexamethasone and 10<sup>-8</sup> cortisol compared with ethanol controls (Table 1).

Because Thrash and Cunningham<sup>7</sup> have shown that dexamethasone stimulates DNA synthesis and division of contactinhibited 3T3 cells we measured the 24 h viral production of HSV-2syn in growing, non-confluent monolayers compared with contact-inhibited 3T3 confluent cell cultures. We found no significant differences in the yields. Thus the increase in viral production seems to be independent of the stimulation of division of contact-inhibited 3T3 cells by glucocorticoids.

In contrast to the results with HSV-2syn and 3T3 cells, when SMF monolayers were infected with HSV-2syn, steroids produced no change in 24 h viral production or plaquing efficiency. Dexamethasone and cortisol did, however, cause a decrease in the size of the plaques (Table 2). The reduction in plaque diameter correlated with a decrease in the ratio of released virus to cell-associated virus 24 h after infection (Table 3).

Since the spread of virus and plaque formation with HSV-

Table 2 Reduction in size of HSV-2syn plaques in SMF with glucocorticoid

			_
Glucocorticoid		Plaque size (mm)	
Dexamethasone	10-5 M	1.05	
Donaine in an a	10 <sup>-6</sup> M	1.15	
	10 <sup>-7</sup> M	1.4	
Control		2.4	
Control			

Plaque diameter figures are averages of measurements of 10 plaques. The plaques were measured by a linear grid inserted in the ocular piece of a dissecting microscope.

2syn are related to both formation of polykaryocytes and release of virus from infected cells we investigated the effects of steroids on infection of SMF by non-syncytial HSV-2. We found no effects on virus yield, released fraction of virus, plaquing efficiency or plaque size. The reasons for this difference between the strains of HSV-2 are not clear, although steroids apparently affect the release of virus from polykaryocytes produced by HSV-2syn in SMF cultures but not from the mononuclear cells releasing HSV-2non-syn.

Table 3 Influence of glucocorticoids on the released fraction of HSV-2syn infecting SMF

Glucocorticoid		Viral yield (PFU		R/CA ratio
		Cell-associated (CA)	Released (R)	
Dexamethasone	10 <sup>-5</sup> M	28.8×10 <sup>5</sup>	$80 \times 10^{5}$	2.7
	10-6 M	28×105	$70 \times 10^{5}$	2.5
	10 <sup>-7</sup> M	36×105	15×105	0.4
Cortisol	10 <sup>-6</sup> M	$18 \times 10^{5}$	$45 \times 10^{5}$	2.5
Control	28 100	5 × 10 <sup>5</sup>	140×105	28.0

<sup>\*</sup> Input 1×105 PFU per flask. Twenty-four hours after infection the supernatant of the cultures was collected and the virus present was titrated as 'released virus'. After removing the supernatant, 2 ml of medium was added to the culture flasks and the intercellular virus was recovered as described in the total viral yield experiments.

Our results show that the effect of glucocorticoids on HSV-2 infection in vitro depend on the type of cell culture as well as the characteristics of the viral strain. In 3T3 cells infected with the syncytial strain of HSV-2, steroids produce increased yields of virus and twofold to fivefold increases in plaquing efficiency. In Swiss mouse fibroblasts, steroids produce no increase in virus yield or plaquing efficiency, but there is a decrease in plaque size and amount of virus released from cells infected with the syncytial strain. The findings suggest that in HSV infections in vivo, in addition to effects on the immune system, steroids act directly on infected cells to either increase susceptibility and yield, or decrease release.

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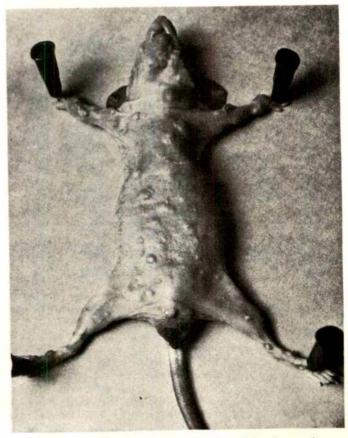
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# Increased susceptibility to virus oncogenesis of congenitally thymus-deprived nude mice

A ROLE of thymus-deprived (T) lymphocytes in controlling tumour development has been widely discussed. The most convincing evidence in support of such a mechanism comes from observations on the induction of tumours by polyoma virus in adult mice1. Normal adult mice do not develop tumours after infection with the virus, but mice deprived of T lymphocytes by thymectomy and repeated inoculations of anti-lymphocytic serum and infected with polyoma virus as adults do develop tumours. If such animals are reconstituted with T lymphocytes from syngeneic immune donors, tumours do not develop.

Congenitally thymus-deprived (nu/nu) mice provide a convenient test system for analysing the role of T lymphocytes in surveillance against tumours. These mice are unusually susceptible to infections by certain viruses and other agents1, and they soon die if maintained in normal laboratory colony conditions. Nude mice can be maintained under special conditions for longer and those surviving for 7 months have not developed tumours spontaneously2. The time of appearance and numbers of tumours in nude mice treated with methylcholanthrene were not significantly different from those in control mice3; the relatively large dose of carcinogen used could have been immunosuppressive, however, and the skin of a nu/nu mouse is clearly abnormal.

These observations have raised some doubts about the role of T lymphocytes in surveillance against tumours. We decided, therefore, to investigate the susceptibility of nu/nu mice to tumour induction by viruses. The nu/nu mice used were bred in this institute on a TO background; 20 male nu/nu mice and 20 littermate control nu/+ mice aged 6-8 weeks were infected with polyoma virus. One half of the nu/nu and control animals were



Nude mouse 8 weeks after infection with polyoma virus, showing multiple skin tumours.

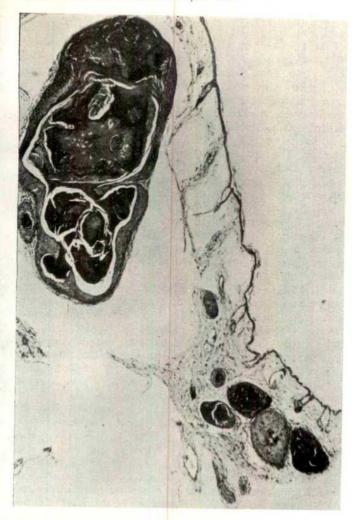


Fig. 2 Section of nude mouse skin, showing one large and several small tumours. (× 550).

injected intraperitoneally with 0.2 ml horse anti-mouse lympho cyte serum (ALS) twice weekly for 8 weeks. No tumours developed in any nu/+ mice. All nu/nu mice given ALS developed skin tumours within 4 weeks. Beginning 4 weeks after infection, untreated nu/nu mice developed skin tumours, and by 12 weeks all had multiple skin tumours (Fig. 1). The tumours were mostly carcinomas of the hair follicles (Fig. 2) and occasionally of sebaceous glands. Some mice also showed widely disseminated bone tumours, involving the femur, tibia, pelvic bones, vertebrae and ribs, as detected by radiological examination. The histological appearance was that of endosteal and periosteal osteosarcoma, the latter infiltrating muscle. Each animal had several bone tumours, mostly small but some as large as I cm in diameter. Other tumours present in the animals included adenocarcinomas of the salivary glands, carcinomas of the renal cortex and flat transitional-cell carcinomas of the renal pelvis.

In another experiment 6 male nu/nu mice and 12 nu/+ littermate control mice aged 8 weeks were inoculated intra-

Table 1 Tumours induced in nu/nu mice inoculated with polyoma virus at 6-8 weeks of age

Skin hair follicular carcinoma Osteosarcoma	% Tumours 100.0
Salivary gland carcinoma	36.4
Carcinoma of kidney	45.6 55.6
Transitional cell carcinoma of rena	pelvis 27.3

106 plaque-forming units of the small-plaque variant of SE polyoma virus inoculated intraperitoneally.

muscularly with 103 ID50 of murine sarcoma virus (MSV/M). None of the heterozygous control animals developed tumours, whereas all nu/nu mice developed large tumours of their thighs and were killed 3 weeks later. They also had marked splenomegaly and histological examination showed lesions in the muscle and thighs expected of murine sarcoma virus\*.

These results show that nu/nu mice are highly sensitive to virus oncogenesis at an age when their heterozygous littermates are resistant, confirming the major role of T lymphocytes in limiting virus oncogenesis. The accelerated development of tumours in recipients of ALS, however, suggests that there may be a second surveillance mechanism which does not involve T lymphocytes, possibly the antibody-dependent effector cell system5. This might account for the low incidence of tumours recorded in nu/nu mice2, although they were observed only for 7 months.

MSV/M was provided by Dr J. J. Harvey.

Note added in proof: Prof. M. Vandeputte, Leuven, Belguim, has independently found that nude mice are highly susceptible to oncogenesis when infected with polyoma virus as adults.

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## Effect of hypophysectomy on a virus-induced T-cell leukaemia

We have found that removal of the pituitary from rats decreases their susceptibility to leukaemia induced by Gross virus. This provides preliminary support for our hypothesis that the pituitary through one of its hormones, is involved in leukaemogenesis.

Gross passage A virus was injected intraperitoneally into newborn Sprague-Dawley rats. At 3 weeks of age the animals were weaned and separated by sex, and approximately half of them were hypophysectomised. At weekly intervals thereafter they were weighed and then a differential and total white blood cell count was performed. Autopsies were conducted after death and leukaemia was diagnosed on the basis of gross and microscopic findings of the presence of typical lymphoblasts in the peripheral blood. Autopsied animals had thymus tumours and other pathology typical of T-cell leukaemia1. Hypophysectomy was considered to have been complete if the animals gained less than 100% of their weight after surgery and the pituitary was absent at autopsy.

By 12 weeks after injection of the virus, the incidence of leukaemia was none out of 11 in hypophysectomised rats, 8 out of 12 in incompletely hypophysectomised rats, and 37 out of 37 in unoperated rats.

Thus hypophysectomy significantly impairs leukaemogenesis induced by Gress virus. It is not clear at the moment why our results conflict with those of Nagareda and Kaplan who found that hypophysectomy did not impair radiation-induced leukaemia in C57BL mice2. In their study the T-cell lymphomas appeared slightly earlier in the operated animals.

The possibility that at least one phase of leukaemogenesis depends on the pituitary suggests new ways of investigating leukaemia in both experimental animals and man. In the case of

a T-cell lymphoma the pituitary may be affecting the thymic epithelium to create the conditions appropriate for leukaemogenesis. It may do this through known hormones, such as growth hormone or prolactin, or unknown hormones such as a thymus-stimulating hormone. Growth hormone which influences thymus development, may be the critical hormone<sup>3</sup>. If so, it probably acts by stimulating the synthesis of somatomedin, perhaps even a somatomedin unique for the thymus. If Gross virus impairs the synthesis of this somatomedin, leukaemia may develop as a consequence of the lack of a feedback inhibitor to suppress pituitary stimulation of the thymus.

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# In vitro sensitisation to dinitrochlorobenzene in guinea pigs

According to Medawar's concept of peripheral sensitisation<sup>1, 2</sup> the induction phase of contact sensitivity may consist of a sequence of three stages. These are the formation of antigen by conjugation of the hapten with skin proteins; the recognition of this antigen by circulating lymphocytes and the activation of these cells passing by the site of antigen application; and the proliferation and differentiation of these activated lymphocytes in the draining lymph node resulting in development of memory and affector cells.

guinea pigs highly purified from granulocytes and macrophages on a glass bead column were incubated in a  $10^{-6}$  M solution of dinitrochlorobenzene (DNCB) in medium 199 containing 10% isologous guinea-pig serum for 3 h at  $37^{\circ}$  C in a 10% CO<sub>2</sub> atmosphere. Suspension of  $5\times10^{6}$  cells per ml medium were used. After removing as much free DNCB as possible by at least four washings,  $3\times10^{7}$ – $5\times10^{7}$  lymphocytes in 0.2 ml medium were injected intradermally into the skin of the left ear of strain-13 recipients. These animals were skin-tested 14 d later with 0.09%, 0.05% and 0.03% DNCB solutions in acetone. The contact reactions were read 24 h later and assessed according to an arbitrary scale from 0 to 3 (ref. 4). The degree of hypersensitivity was expressed as the total of all three readings in each animal.

In the first group donors and recipients were normal strain-13 guinea pigs. In the second group the possible active sensitisation of the recipients by the hapten conjugated to lymphoid cells was excluded by using tolerant guinea pigs as recipients. Tolerance to DNCB was induced by two intravenous injections of dinitrobenzenesulphonic acid sodium salt (DNBSO<sub>3</sub>) 28 d and 14 d before the transfer. It has been shown that tolerance induced by this method is complete and permanent and cannot be broken even by using dinitrofluorobenzene (DNFB) in FCA for sensitisation (group 5). In the third group, allogeneic peritoneal exudate lymphocytes from Himalayan spotted white guinea pigs were used as donor cells assuming that rejection of these cells will prevent the adoptive sensitisation of recipients having no effect on a possible active sensitisation with hapten-lymphocyte conjugate. In the fourth group, lymphocytes from tolerant donors incubated with the hapten were transferred to normal syngeneic recipients.

The results are summarised in Table 1. It is evident that peritoneal exudate lymphocytes treated *in vitro* with a nontoxic solution of DNCB are capable, when transferred to normal syngeneic recipients, of inducing contact sensitivity to this hapten, as demonstrated by a positive epicutaneous test 14 d after the transfer. This effect could be the result of either sensitisation of the lymphocytes *in vitro* or the formation of a strongly immunogenic antigen by conjugation of DNCB with lymphocytes, as shown by Baumgarten and Geczy<sup>5</sup>, Asherson

Table 1 Sensitisation of guinea pigs by intradermal injection of in vitro DNCB-treated lymphocytes

Cen		nors Recipients			No.	lo. Contact	Sensitised/Total
Grou	Strain	Immune status	Strain	Immune status		test	,
1 2 3 4 5	13 13 Himalayan 13	Normal Normal Normal Tolerant	13 13 13 13	Normal Tolerant Normal Normal Tolerant* Controls	6 16 5 7	$\begin{array}{c} 1.6 \pm 1.3 \\ 0.9 \pm 0.7 \\ 0 \\ 1.4 \pm 0.2 \\ 0 \end{array}$	4/6 12/16 0/5 7/7 0/10

<sup>\*</sup> Tolerant guinea pigs not receiving cells but injected with 500 µg DNFB in FCA and tested 14 d later.

Macher and Sommer<sup>3</sup> failed to demonstrate convincingly peripheral sensitisation of lymphocytes in their combined in vitro-in vivo model using outbred guinea pigs, but their results do not exclude this concept either. Their main difficulty was to distinguish between active sensitisation with hapten-conjugated lymphocytes and transfer of sensitivity with cells already sensitised in vitro.

Here in our study, a similar model was used to solve this important problem. As tolerant guinea pigs cannot become sensitised by application of the hapten but are adoptively sensitised by cells from hypersensitive donors, immunocompetent lymphocytes, when activated in vitro, should also induce contact sensitivity in tolerant recipients.

Peritoneal exudate lymphocytes from inbred strain-13

et al.6 and McFarlin and Balfour?. This second possibility was excluded, however, in the experiments in groups 2 and 3.

It was demonstrated that guinea pigs rendered tolerant by two intravenous injections of DNBSO<sub>3</sub> cannot become actively sensitised even when a relatively high dose of hapten (500 µg DNFB) in FCA is used for sensitisation (group 5) and epicutaneous tests were repeated for a period of more than 1 yr. The tolerant animals become hypersensitive, however, by adoptive transfer of lymphoid cells from hypersensitive donors<sup>8</sup>.

It was shown recently that tolerance in contact sensitivity may be caused by specific suppressor cells preventing the proliferation of normal immunocompetent lymphocytes, probably at a very early stage. Our results in group 2, showing that 12 out of 16 tolerant recipients of lymphocytes preincubated with DNCB in vitro became sensitised, indicate that these cells already reached a stage when they cannot be inhibited by the suppressor cells any more. These results favour the concept of in vitro sensitisation or at least activation of specific immunocompetent lymphocytes. The higher degree of sensitivity of normal recipients in comparison to tolerant ones might be caused by additional active sensitisation of normal animals, but not of tolerant ones, with a conjugate of DNCB with lymphoid cells<sup>5-7</sup>.

The idea that lymphocytes can actually be sensitised in vitro is further supported by the finding that allogeneic cells (group 3) as well as non-viable autologous cells3 have no sensitising capacity, although their capacity to form immunogenic conjugates may not be altered.

The results in group 4 do not necessarily contradict the hypothesis of peripheral sensitisation; they need, however, a further explanation. One possibility is that the lymphocyte suspension from tolerant donors may either not contain suppressor cells or these cells are not able to act in vitro and prevent immunocompetent cells from becoming activated by preincubation with the hapten.

As it is known that guinea pigs can become sensitised to DNCB conjugated to different homologous skin and serum proteins10, and therefore also to DNP-conjugates formed with proteins of the culture medium, the present results might be regarded as evidence that the first two steps of the induction phase of contact sensitivity, namely formation of the antigen and its recognition by immunocompetent cells, can be accomplished also in vitro. Lymphocytes incubated with DNCB in vitro become activated beyond a stage where they cannot be inhibited any more by suppressor cells. An intradermal injection of these cells acts somehow as an adoptive sensitisation. An additional active sensitising effect of a DNCB-lymphocyte conjugate, however, may not be excluded when normal recipients are used. This may explain the higher degree of sensitisation in normal recipients than in tolerant ones.

As the sensitisation of tolerant recipients can be achieved only by transfer of already specifically activated lymphocytes, we assume that in our experiments cells incubated with the hapten in vitro were actually sensitised. The fact that in our in vitro-in vivo model the initial steps of sensitisation are accomplished in vitro may be regarded as support for the concept of peripheral sensitisation.

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# Large numbers of cells in normal mice produce antibody components of isologous erythrocytes

THERE are a number of arguments against the idea' that self-tolerance depends on a purge of all self-reactive ciones early in development. First, antigens which appear later in development, for example, Ig idiotypes, must induce tolerance to themselves. Second, autoimmune diseases are often reversible, implying that when anti-self-reactive cells arise late, they may still be controlled. Third, the recent finding (refs 2-4 and L. M. Pilarski and A.J.C., unpublished) that single clones of antibody-forming cells rapidly produce variants also implies a continuous monitoring and control of immunocompetent cells. Self-reactive variants must arise during many immune responses, and must normally be prevented from causing autoimmune disease. Fourth, the distinction between anti-self and anti-not-self specificities is obviously not clear-cut. Given the great diversity of self components in mammals, it is probable that almost any antibody will react to some self antigens with low affinity.

These considerations suggest that normal animals may contain many auto-reactive cells which are, however, prevented from causing autoimmune disease by some control mechanism. Here I demonstrate plaque forming cells (PFCs) against components of autologous erythrocytes (RBCs) in normal mice.

Although others have reported the presence of small numbers of plaques on normal isologous RBCs\*-7, I could not find such PFCs in spleens of 8-week-old CBA mice. When their RBCs were first treated with any of the proteolytic enzymes trypsin, papain or bromelain, however, several thousand PFCs were found per spleen in normal mice of this and other strains (Table 1). Bromelain was used throughout the rest of this study: it is an enzyme which has been fairly widely used8-12 to demonstrate allo- and autoantibodies against human RBCs. The mouse RBCs were first incubated as a 50% suspension with bromelain at a final concentration of 10 mg ml-1 in phosphate buffered saline for 30 min. The RBCs were washed three times and then incubated at 37°C for 1 h with spleen cells in slide assay chambers13. Rabbit serum as a complement source gave about three times as many, and larger, plaques than normal guinea-pig serum. Only direct plaques were found -a guinea-pig anti-mouse Ig failed to increase the number of plaques at any dilution. They were up to about 0.5 mm in diameter, with many very small plaques.

A number of control experiments confirmed that these plaques were caused by release of Ig from a single central cell. In summary: the plaques did not appear in the absence of complement, or at 4°C, and they were inhibited by guinea-pig and anti-mouse-Ig antiserum to exactly the same extent as mouse anti-sheep RBC plaques, over a range of concentrations of antiserum. At high dilutions of spleen cells a single central cell could be seen in each plaque, and six of these PFCs, when isolated by micromanipulation<sup>14</sup> and stained looked like the basophilic mononuclears which make conventional anti-sheep RBC plaques15. Plaques were not affected by preabsorbing the rabbit complement with bromelain-treated mouse RBCs, showing that they were not caused by a reaction between secreted mouse protein and a cytophilic rabbit-anti-mouse antibody on the treated red cells. They were not caused by nonspecific (Fc) adherence of secreted Ig to treated RBCs as immunisation with sheep RBCs which produced appsoximately 10° anti-sheep PFCs per spleen, did not increase the number of plaques on bromelain-treated mouse RBCs. Also high concentrations of normal mouse serum in the assay mixture did not inhibit plaque formation.

Table 1 Numbers of plaques against bromelain-treated CBA mouse RBCs in various lymphoid tissues

Species and strain of		No. of animals	ment.	PFCs per 108 nucleated cells
animal	Age	tested	Tissue	arithmetic mean (+range)
CBA mice*	8-10 weeks	42	Spleent	5,320 (960-15,840)
CBA mice‡	8-10 weeks	4	Spleen	1,840 (1,280–2,480)
C57BL •	8-10 weeks	4	Spleen	940 (480–1,280)
BALB/c	8-10 weeks	4	Spleen	1,020 (880–1,120)
$(CBA \times C57BL) F_1$	8-10 weeks	4	Spleen	2,200 (640-3,440)
NZBII	8-10 weeks	4	Spleen	4,900 (1,440-10,400)
Nude (nu/nu)	8-10 weeks	4	Spleen	560 (80-1,520)
CBA mouse§	8-10 weeks	3	Bone marrow	2,600
· ·			Thymus	100
			Mesenteric	
			lymph node	600
CBA mouse	56 d	6	Spleen	33 (15-70)
	9–11 d	8	Spleen	229 (30-900)
	21 d	6	Spleen	2,317 (600-5,200)
	6 months	3	Spleen	2,400 (1,120-4,000)
Lewis rat	3 months	5	Spleen	1,560 (750-2,540)
BALB/c mice¶	8 weeks	6	Spleen	1,400 (680–1,920)
CBA mice**	8-10 weeks	4	Spleen	206,000 (147,000-272,000)

<sup>\*</sup> This is a summary of many separate experiments.

CBA mice were treated in various ways to try and increase the number of PFCs in their spleens against isologous erythrocytes which had been incubated with bromelain. The following treatments did not increase the number of plaques significantly over a period of one week: injection of allogenic red cells or spleen cells, injection of isologous cells in Freund's complete adjuvant, a single injection of 10° rat RBCs. A dramatic rise in the number of plaques was, however, induced by a single intraperitoneal injection of 50 µg of lipopolysaccharide (LPS) from Escherichia coli strain 0128:B12 (Table 1). These plaques peaked at 2-3 d then returned abruptly to normal background levels (in preparation).

Against what antigens are these antibodies directed? There are two broad possibilities: either they react with entirely new antigenic determinants created by the enzyme treatment, or they are directed against antigens which are present within the normal RBC. The first alternative seems

to be ruled out by two findings. First, bromelain-treated mouse RBCs provoke little or no increase in the number of plaques on similar target cells when injected into mice (Table 2). In other words, the enzyme treatment has not converted mouse RBCs into grossly foreign particles. Second, rats injected with normal mouse red cells respond by producing both increased numbers of PFCs (Table 2) and high antibody titres (Table 3) against bromelain-treated mouse RBCs. This shows that the antigens on the surfaces, of such treated cells do form part of the normal mouse RBC.

What does the bromelain treatment do to the RBCs to allow mouse lymphoid cells to cause their lysis? Again there seem to be two possibilities. The enzyme may make the RBCs more fragile and able to be lysed by lower affinity, or fewer, autoantibodies. Alternatively, the bromelain may expose some component(s) normally not available to the antibody, which are, however, revealed during normal

Table 2 Numbers of PFCs in the spleens of mice and rats injected with normal or bromelain-treated mouse RBCs

Experiment No.	Test animals species (+No.)	RBCs injected	Days after injection	PFCs per spleen ( BRBCs*	±s.e.) against NRBCs*
1	CBA mice (3)	3×108 BRBCs*	4†	$4.056 \pm 80$	NT‡
	CBA mice	3×106 BRBCs	4	$4.064 \pm 928$	NT
	CBA mice	$3 \times 10^8$ NRBCs	4	$4,076 \pm 557$	NT
2	CBA mice (3)	2×109 BRBCs	3	$9,812 \pm 1,768$	NT
	CBA mice	2×10° NRBCs	3	2,094 + 346	NT
	CBA mice	2×109 BRBCs	6	$4.108 \pm 504$	NT
	CBA mice	2×10° NRBCs	6	$2,508 \pm 509$	NT
3	Lewis rat No. 1 •	• 6×108 NRBCs	4	41,600	89,600
	Lewis rat No. 2	6×108 NRBCs	4	19,680	32,000
	Lewis rat No. 3	6×108 NRBCs	4	66,560	126,720

In experiment 1, RBCs were injected intravenously, and in experiments 2 and 3, intraperitoneally.

<sup>†</sup> Plaques were counted in a fraction of a spleen cell suspension (usually 1/40). The total number of cells per adult mouse spleen has been assumed to be 10<sup>8</sup> throughout this table.

<sup>‡</sup> These four mice, and the mice of five other strains below it, were all assayed on the same day against the same preparation of bromelain-treated mouse RBCs. The sensitivity of the assay appeared to vary from day to day, and was low on this occasion. There was no significant difference between plaque numbers on isologous or allogeneic treated RBCs.

<sup>§</sup> A pool of tissues from three 8-week-old mice was tested in each case.

<sup>||</sup> In a separate experiment, three 6-month-old NZB females had 9,100, 12,000 and 22,400 PFCs per spleen.

<sup>¶</sup> These mice were taken from strictly isolated germ-free conditions.

<sup>\*\*</sup> Assayed 2 d after 50 µg LPS (intraperitoneal injection).

<sup>\*</sup> BRBC, Bromelain-treated CBA mouse RBC; NRBC, normal CBA mouse RBC.

<sup>†</sup> In this experiment there was also no significant response on days 1, 2 or 7 after injection.

<sup>‡</sup> NT, Not tested. In similar experiments where spleens from normal mice or mice immunised with normal or bromelain-treated RBCs were tested, no plaques were found on normal target RBCs.

breakdown of the erythrocytes either in foreign species (rats) or in mice. This latter explanation is supported by results of absorption of serum from rats immunised with normal mouse RBCs (Table 3). Absorption with bromelaintreated mouse RBCs preferentially lowered the titre to similarly treated target RBCs without much affecting the titre to normal mouse RBCs, and vice versa. This seems to show that anti-bromelain-mouse RBCs and anti-normalmouse RBC antibodies are different populations. Similarly, rats immunised with normal mouse RBCs had more plaques on normal than on bromelain-treated mouse RBCs, again showing that the treated cells are not merely a more sensitive indicator of all antibody which lyses normal cells; they seem to have different antigenic surfaces.

Attempts to demonstrate plaques on isologous RBCs in chicken and sheep have yielded none so far with either normal or bromelain-treated RBCs. Normal rats had less than 30 PFCs per spleen on treated or untreated rat RBCs, but approximately 8,000 PFCs were found in the spleens of LPS-stimulated rats tested against bromelain-treated rat RBCs. It is interesting that rats do have a high normal plaque level to bromelain-treated mouse RBCs (Table 1): possibly mouse and rat RBCs share common internal antigens which are more difficult to reveal by enzyme treatment of the rat RBCs.

The numbers of plaques against bromelain-treated isologous RBCs in the spleens of normal mice is more than an order of magnitude higher than the 'background' level described by many authors against highly immunogenic foreign erythrocytes. This suggests an ongoing response to breakdown products of autologous RBCs, producing antibody which may assist removal of effete cells. The idea that the antigen comes from within rather than from crossreactive environmental stimuli is reinforced by the fact that germ-free mice had similar plaques (Table 1).

Table 3 Absorption of rat-anti-mouse RBC serum with normal or bromelain-treated mouse RBCs

Serum pool	Days after injection*	Absorbed with†	Titre‡ against mouse RB Normal Bromelain-treat		
	•		TOTTILL	Bromeiam-treated	
1	4	- MANAGAMAN	256	256	
		Normal RBCs	< 8	256	
		Bromelain RBCs	128	< 8	
2	6		1.024	4,048	
		Normal RBCs	32	760	
		Bromelain RBCs	256	32	
3	7	Waterlands .	128	512	
		Normal RBCs	< 8	256	
		Bromelain RBCs	128	< 8	

\* Each serum is from a pool of three Lewis rats injected intraperitoneally with mouse RBCs. Antigen dose was  $6 \times 10^8$  RBCs for sera 1 and 3, and  $2 \times 10^9$  for serum 2.

Absorptions were done by mixing 0.5 ml of serum, diluted 1:8, with 0.2 ml of normal or bromelain-treated mouse RBCs. The mixture was incubated for 15 min at 37° C, then the RBCs removed

by centrifugation. This was repeated twice.

‡ Titres are reciprocals of 50% haemolytic and point, using rabbit complement (final dilution 1:20) and RBCs at a final dilution of 0.5%.

This work raises two further questions about tolerance to autologous erythrocytes. First, why is tolerance to the surface of untreated RBCs so much more 'complete', in that relatively few plaques can be found on such targets? This may depend on an earlier confrontation between 'external' RBC antigens and the developing host immune system. The life span of mouse RBCs is 20-45 d (ref. 16), so large numbers of degenerating cells might not be available to act as antigen until 1-2 weeks after birth, while intact RBCs would be present much earlier. Second, why do plaque

numbers stabilise at about 5,000 per spleen (in adult CBA mice) when there is presumably ample antigen available, as RBCs degenerate, to push the response still higher? Even large doses of bromelain-treated RBCs will not increase this number much (Table 2). It seems possible that an equilibrium is maintained between constant antigen stimulation and some suppressor mechanism. This view, which has important implications for self-tolerance, is supported by recent experiments (data in preparation) showing that injection of normal mice with antilymphocyte serum will increase the numbers of plaques in their spleens against bromelain-treated isologous RBCs.

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Note added in proof: Since submitting this paper I have read the article by Detleer and Edginton (Clin. exp. Immunol., 16, 431; 1974) which describes similar numbers of PFC on bromelain-treated mouse erythrocytes from NZB mice.

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# Inhibition of rat lymph node cell cytotoxicity by hepatoma-associated embryonic antigen

One feature frequently accompanying malignant transformation is the re-expression of embryonic characteristics, and the presence of tumour-associated embryonic antigens has been demonstrated on a wide range of experimental and human tumours1-5. Studies in our laboratory, using aminazo-dyeinduced rat hepatomas, have shown that these re-expressed antigens are detectable on cells derived from 14-16-d-old rat embryos, but not cells from adult rat tissue1,8 and in vitro microcytotoxicity tests have revealed that rats bearing transplanted hepatomas exhibit a cell-mediated immune reaction against the embryonic antigen (EA)6.

Here we present results which demonstrate that embryonic antigen isolated from two rat hepatomas is capable of inhibiting the cytotoxicity of lymphocytes specifically sensitised to

Table 1 Effect of hepatoma D23 associated embryonic antigen on the *in vitro* cytotoxicity of multiparous and tumour-immune rat lymph node cells

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Test	Target cells	Status of LNC donor	LNC pretereated with D23 EA*	Mean no. target per well (± s.e.) Normal LNC	cells surviving after exposure to: Test LNC	2% Lymphocyte cytotoxicity	Inhibition
1	• D23	Multiparous	12.5	$\begin{array}{c} 28 \pm 4 \\ 31 \pm 5 \end{array}$	$\begin{matrix} 7 \pm 1 \\ \textbf{30} \pm \textbf{2} \end{matrix}$	75¶ 3	<del>-</del> 96§
1 1923	D23-immune	12.5	$\begin{array}{c} 28 \pm 4 \\ 31 \pm 5 \end{array}$	$\begin{array}{c} 9\pm2\\ 7\pm1 \end{array}$	68¶ 77¶	-13	
2	2 D23	Multiparous	5 10	$78 \pm 1$ $73 \pm 8$ $64 \pm 7$	51 ± 7 59 ± 5 60 ± 6	35‡ 19 7	46 80‡
2	<i>D23</i>	D23-immune	10	78 ± 1 64 ± 7	59 ± 8 45 ± 5	24‡ 30†	<b>-25</b>
3	D23	Multiparous	15 30	$\begin{array}{c} 22  \pm  2 \\ 23  \pm  2 \\ 22  \pm  2 \end{array}$	$9 \pm 1$ $11 \pm 1$ $17 \pm 1$	59¶ 52¶ 23‡	12 61‡
4	D23	Multiparous	15 30	$\begin{array}{c} 22\pm2 \\ 23\pm2 \\ 22\pm2 \end{array}$	$     \begin{array}{c}       6 \pm 1 \\       8 \pm 1 \\       11 \pm 3     \end{array} $	73¶ 65¶ 50§	11 32†
5	D23	Multiparous	15 30	$\begin{array}{c} 26 \pm 2 \\ 26 \pm 2 \\ 32 \pm 4 \end{array}$	$\begin{array}{c} 12 \pm 2 \\ 12 \pm 1 \\ 23 \pm 5 \end{array}$	54¶ 54¶ 28	
6	14-d embryo	Multiparous	15 30	$\begin{array}{c} 25 \pm 1 \\ 26 \pm 3 \\ 26 \pm 2 \end{array}$	17 ± 3 16 ± 2 26 ± 3	32‡ 38‡ 0	$-\frac{19}{100}$

<sup>\*</sup> Hepatoma D23 associated embryonic antigen added per  $5 \times 10^5$  LNC (µg protein). † P = <0.1 ‡ P = <0.05 § P = <0.01 ¶ P = <0.001.

embryonic antigen component against hepatoma cells *in vitro*. This mechanism is similar to that shown for the individually distinct hepatoma-specific antigen<sup>8</sup>. The relevance of our findings to the modification of tumour growth *in vivo*, and to antigenic expression on human tumours is also discussed.

Our investigation was designed to evaluate whether in a model *in vitro* system, hepatoma-associated EA had the capacity to interfere with lymphocytotoxic reactions. For this purpose, EAs were isolated following sequential fractionation of the soluble cytoplasmic protein fraction of homogenates from two transplanted hepatomas (D23 and D30) as previously described<sup>1,7</sup>. These procedures make possible the recovery of an antigenic fraction which by conventional criteria seems homogeneous although most recent studies indicate that this material represents at least two distinct, but co-purifying, macromolecules; one being a protein component of molecular weight between 65,000 and 70,000 and the other being a glycoprotein of lower molecular weight (M.R.P., and Z. Tökés, unpublished). The

final EA preparations from hepatomas D23 and D30 retain the capacity to neutralise the membrane immunoflurescence staining of viable tumour cells by multiparous (MP) rat sera which have been shown to contain antibody directed against cell surface-expressed EA<sup>1,7</sup>.

Hepatoma D23 and D33 cells were cultured as monolayers in Eagle's minimal essential medium (MEM)+10% calf serum, and cells obtained following trypsinisation of whole 14-d-old rat embryos were grown in Waymouth's medium+10% foetal calf serum. For lymphocytotoxicity tests, tumour or embryo cells were plated in the wells of Microtest II plates (Falcon) at a concentration of 100-200 cells per well in Eagle's MEM+10% calf serum, and incubated for 24 h at 37° C to allow adherence. MP lymph node cells (LNC), obtained from the cervical, axillary and mesenteric lymph nodes were taken from rats having had three or more pregnancies and which were pregnant at the time of assay. Tumour-immune LNC were obtained from rats which had received three or more  $\gamma$ -irradiated (15,000 rad)

Table 2 Effect of hepatoma D30 associated embyronic antigen on the in vitro cytotoxicity of multiparous rat lymph node cells

			-	•		
Test	Target cells	LNC pretreated with D30 EA*		t cells surviving after exposure to: MP LNC	% Lymphocyte cytotoxicity	// Inhibition
1	D23	20 40	33 ± 4 40 ± 6 39 ± 4	$16 \pm 3$ $21 \pm 4$ $32 \pm 5$	52‡ 48† 18	8 63†
2	D23	50	41 ± 6 42 ± 6	29 ± 2 42 ± 5	29† —2	107†
3	D33 •	50	$\begin{array}{c} \textbf{350} \pm \textbf{51} \\ \textbf{384} \pm \textbf{49} \end{array}$	$38\pm9 \ 86\pm15$ .	89§ 78§	12
4	D33	50	350 ± 51 384 ± 49	$55 \pm 10$ $119 \pm 32$	84§ 69§	18

<sup>\*</sup> Hepatoma D30 associated embryonic antigen added per 5  $\times$  10<sup>5</sup> LNC (µg protein). †  $P = < 0.05 \ddagger P = < 0.01 \S P = < 0.001$ 

hepatoma D23 grafts, followed by challenge with  $5 \times 10^5$  viable tumour cells at monthly intervals. Aliquots (0.4 ml) containing  $5 \times 10^6$  LNC were incubated at  $37^{\circ}$  C for 60 min with 0.2 ml volumes of EA at various concentrations or in control tests, with medium alone (0.2 ml aliquots MEM). LNC were sedimented at 120g for 5 min and suspended in 2 ml MEM.  $5 \times 10^5$  LNC (0.2 ml volume) were added to each of eight wells in Microtest II plates and after 60 min foetal calf serum was added to a concentration of 10% in each wall. After incubation for 48 h at 37° C any remaining adherent tumour cells were washed, fixed, stained with Gentian violet and counted.

Table 1 (tests 1-5) illustrates the effect of pretreating MP or D23-immune LNC with hepatoma D23 EA. Previous incubation of 5×10<sup>5</sup> MP LNC with 12.5 µg D23 EA reduced their cytotoxicity from 75 to 3\%, representing a 96\% inhibition of cytotoxicity (Table 1, test 1). This concentration did not, however, reduce the cytotoxic effect shown by LNC from hepatoma D23-immune animals for plated hepatoma cells (Table 1, test 1). Comparable results were obtained in a subsequent experiment (Table 1, test 2) again indicating that D23 EA, at concentrations which inhibit MP LNC cytotoxicity does not affect the cytotoxicity of D23-immune LNC. These findings give further evidence demonstrating that the tumourspecific antigen, present on these aminoazo dye-induced rat tumours and against which tumour-immune LNC exert their cytotoxic effect, is a distinct component from the tumourassociated EA. Tests 3-5 (Table 1) exemplify further data showing the inhibitory effect of D23 EA on the cytotoxicity of MP LNC, and in test 6 (Table 1) the cytotoxicity of MP LNC for 14-d rat embryo cells is also reduced following previous exposure to hepatoma D23 EA.

In comparable tests, the EA preparation from another transplanted hepatoma (D30) was found to display similar activity to the EA isolated from hepatoma D23 (Table 2). Pretreatment of  $5 \times 10^5$  MP LNC with 40-50 µg of hepatoma D30 EA significantly inhibited their cytotoxicity for D23 target cells (Table 2, tests 1 and 2). This finding, indicative of a cross-reactive nature between hepatoma D23 and D30 EA, is also supported by previous studies showing that the isolated EA preparations had the capacity to neutralise the membrane immunofluorescence staining by MP sera of surface antigens expressed on either D23 or D30 target cells<sup>1,7</sup>. A variability in EA expression or specificity, however, was indicated in further experiments using MP LNC pretreated with hepatoma D30 EA (Table 2, tests 3 and 4). In these tests, the cytotoxicity of MP LNC for plated hepatoma D33 cells was not significantly reduced by previous exposure to D30 EA at a concentration of 50  $\mu g$  per 5  $\times$  10<sup>5</sup> LNC.

Previous studies have established the existence of two distinct neoantigens expressed at the cell surface of chemicallyinduced hepatomas. These antigens have been differentiated into a tumour-specific component and an embryonic component by several criteria<sup>1,9</sup>. Thus, MP rat serum was shown to block, at the level of the target cell, MP LNC cytotoxicity for hepatoma cells, but was not capable of blocking tumourimmune LNC cytotoxicity for target hepatoma cells. The reactivity of tumour-immune rat LNC for 14 d embryo cells was, however, effectively prevented by MP serum. These results suggest that the host's cellular immune reponse is manifested by two separate populations of sensitised lymphocytes. In our study a similar differentiation between the two neoantigens is shown; MP LNC cytotoxicity for hepatoma cells was inhibited at the effector cell level by hepatomaassociated EA, but this treatment did not affect the cytotoxicity of tumour-immune LNC for hepatoma cells. The involvement of hepatoma-associated EA in eliciting a tumourrejection response is less clear. Immunisation of rats with foetal tissue failed to induce protection against low dose hepatoma cell challenge10. Similar results have been found using other tumour systems11,12, although embryo immunisation has been shown to induce protection against SV40-induced hamster tumours13,14 and 3-methylcholanthrene-induced mouse, rat

and guinea pig sarcomas<sup>15-17</sup>. The failure of embryonic antigens to induce an effective tumour-rejection reaction may be due to the stability, and/or transient appearance of the antigen on the embryo cell surface<sup>1,3,13</sup>; alternatively the route of administration together with the immunising regime and effective EA dosage may greatly influence the eventual immunological response.

Our findings are relevant to studies on human tumours as the embryonic antigens detected by microcytotoxicity tests on carcinogen-induced rat mammary<sup>6</sup> and colonic carcinomas<sup>18</sup> as well as spontaneously arising rat mammary carcinomas and sarcomas6 show organ-type specificities comparable with those identified on many human tumours19 and by analogy the latter may also be re-expressed embryonic antigens. It has further been demonstrated with a limited number of human tumours that antigen mediated inhibition of lymphocyte cytotoxicity may be one mechanism by which a growing tumour evades host immunological control<sup>20,21</sup>. Recent studies showing the presence of hepatoma-associated EA in the serum of tumour-bearer rats implies that such factors may be capable of modifying lymphocytotoxic reactions in vivo (R.C.R., M.R.P., R.W.B., and L.P.S., unpublished), as demonstrated here using in vitro tests.

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# Association of mammalian cell death with a specific endonucleolytic degradation of DNA

MAMMALIAN cell death, observed as deterioration of cellular structure or function, has not been characterised at the molecular level. We report here that human and rodent cells treated with several diverse agents which cause cell death as measured by other criteria, had similar distinct patterns of DNA degradation as assayed by alkaline sucrose gradient techniques. The degradation results from cellular metabolism, not from the initial damage produced by the agents. The similarity of the patterns in our results and those of others in a wide range of cell systems suggests that this degradation may be a general mechanism concomitant with, if not a precedent of, mammalian cell death.

Tritiated thymidine (TdR) is a toxic agent when incorporated into the DNA of mammalian cells<sup>1,2</sup>. The soft B radiation of tritium disintegration has a range of about 1 µm and damages primarily the DNA and its immediate molecular environment. The time course of this toxic action as indicated by cell growth kinetics in monolayers of Chang's liver cells (LICH)3 and Chinese hamster lung cells (CHL)4 is shown in Fig. 1. As the growth rate of monolayer cultures thus treated with 3H-TdR slowed down and the cell density eventually declined, intact cells floated into the medium above the attached cells and could be recovered by centrifugation. These floating cells. though normal in appearance by phase microscopy, had a plating efficiency approximately 1% of that of attached cells. After 24 h of exposure to 3H-TdR, LICH cells floating in the medium incorporated only 13% of 14C-leucine and 32% of <sup>14</sup>C-uridine compared with attached cells for a 2-h pulse.

Sedimentation profiles of DNA from floating and attached cells differed (Fig. 2b). The latter was similar to the profiles reported for diploid and aneuploid cell lines<sup>5-13</sup>, and identical to control profiles of LICH and CHL cells (Fig. 2a and e). They consist of a unimodal homodisperse peak estimated by various authors to be between 125S and  $180S^{5-9}$  which, if composed of single-stranded molecules, would correspond to a weight of between  $1 \times 10^8$  and  $5 \times 10^8$ .

The DNA profile of the floating cells, however, was bimodal with a smaller peak between fractions 4 and 6 ( $\sim$  50S) (Fig. 2b). The amount of DNA found in the degraded peak varied with time and metabolic activity. Identical cultures of LICH cells were labelled for 24 h with 0.5  $\mu$ Ci ml <sup>-1</sup> <sup>3</sup>H-TdR and then incubated in either complete medium (CM) or phosphate buffered saline (PBS) for 8 h. This PBS treatment does

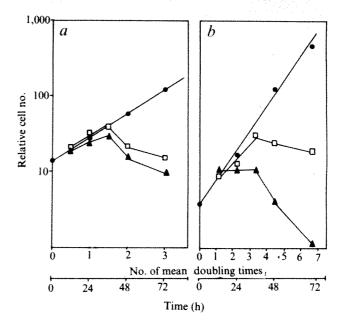


Fig. 1 Relative number of LICH (a) and CHL (b) cells growing in attached monolayers after continued exposure to 0.5 ( ) or 5.0 ( )  $\mu$ Ci ml<sup>-1</sup> of tritiated thymidine (methyl-³H-TdR, 6-15 Ci mmol<sup>-1</sup>) in Eagle's minimum essential medium supplemented with 10% heat inactivated calf serum (LICH) or 15% foetal calf serum (CHL). At times indicated cells were suspended using trypsin and counted using a haemocytometer. •, Control.

not produce toxicity or degraded DNA. Cells were then exposed to fresh medium and incubated at  $37^{\circ}$  C. DNA sedimentation profiles of the floating cells appearing in these cultures were determined 24 and 48 h later. At both times, the DNA of floating cells from cultures whose metabolism was suppressed for 8 h with PBS was less degraded than those incubated in complete medium (Fig. 2c and d). In both cases, however, degradation was more extensive after 48 h than 24 h.

Treatment with toxic agents whose initial action is believed to affect the plasma membrane produced DNA degradation in CHL monolayer cultures. Attached CHL cells, labelled at low  $^3$ H-TdR levels which do not affect growth kinetics, then treated with hypotonic shock, 0.5% deoxycholate or specific CHL antiserum, displayed degradative patterns similar to those induced by  $\beta$  radiation but after shorter post-treatment incubation (Fig. 2f-h). After 5 min of hypotonic shock or deoxycholate treatment, immediate lysis showed the DNA of treated cells was intact, but incubation at  $37^{\circ}$  C in

Cells	Nature of insult	Presumed site of initial insult	Time after insult degradation observed	Reference
LICH	<sup>3</sup> H-decav	DNA	(h) 24	This report
LICH	<sup>3</sup> H-decay	DNA	24	14
CHL	Specific antibody	Cell membrane	0.5	10
CHL	Specific antibody	Cell membrane	Ĩ	This report
CHL	Hypotonic shock	Cell membrane	1	This report
CHL	Detergent	<ul> <li>Cell membrane</li> </ul>	1	This report
CHL nuclei	Nuclei isolation	Nücleus	0.5	Nagle and Belli (unpublished dat
Progeric fibroblasts	<sup>3</sup> H-decay	DNA	4	11
Human lymphocytes	External X rays	Cell	0.5	12
Beagle neurones •	External X rays	Cell	10	5
Rabbit retina	External X rays	Cell	10	6
Mouse thymocytes	External X rays	Cell	0,75 (in vitro) 3.5 (in vivo)	13

complete medium for 60 min and 55 min, respectively, produced extensive degradation (Fig. 2f and g). Similarly, the DNA of cells treated for 60 min at 37° C with medium containing a 1:500 dilution of specific CHL antiserum after the method of Shipley et al.10 was also degraded into similar patterns.

Patterns of DNA degradation similar to those in Fig. 2 have been observed in various mammalian cell populations exposed to several toxic agents. Table I lists observations of DNA degradation in experimental systems described here and similar patterns observed by others. Several important conclusions can be drawn: (1) DNA is degraded in both diploid and aneuploid cells after trauma; (2) the decay of <sup>3</sup>H-TdR is not necessary for degradation since Lett et al.<sup>5</sup> and Wheeler et al.6 used non-dividing, unlabelled diploid cells;

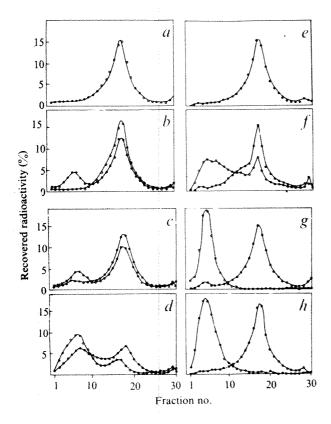


Fig. 2 DNA sedimentation patterns for cells which have received treatment with various toxic agents with and without post-treatment incubation. Cells attached to glass or plastic Petri dishes were labelled with tritiated thymidine (methyl-3H-TdR, 6-15 Cimmol-1, New England Nuclear Corp.), and either treated in monolayer or after suspension by trypsin treatment. After suspension, 2,500-10,000 cells were carefully layered into a lysing solution (0.2 ml, 0.45 N NaOH, 0.5 N NaCl, 0.1 M Na<sub>2</sub>EDTA) on top of preformed 5-20% alkaline sucrose gradients (4.8 ml, 0.1 N NaOH, 0.9 N NaCl, 0.003 M Na<sub>2</sub>-EDTA). After lysing for 4-6 h samples were centrifuged for 15 min at 5,000 r.p.m. followed by 36,000 r.p.m. for 60 min (Spinco 50.1 rotor) at 12-15° C. Gradients were pumped off from the top in 10-drop fractions on to 30 glass fibre filters and counted in a toluene-base scintillation fluid. a-d, LICH cells: a, control LICH cells incubated with 0.1 µCi ml<sup>-1</sup> <sup>3</sup>H-TdR for 16 h; b-d, LICH cells incubated with 5.0 μCi ml<sup>-1</sup> for 24 h; b, with 24 h post-label incubation in complete medium—(•) detached floating cells; ( $\bigcirc$ ) attached monolayer cells—c, detached floating cells with 24 h post-label incubation—( $\bullet$ ) 8 h incubation with phosphate-buffered medium (PBS) followed by 16 h in complete medium or ( ) complete medium (CM) only—d, detached floating cells with 48 h post-label incubation—( ) 8 h incubation with PBS or ( ) CM only. e-h, CHL cells incubated with 0.25 µCi ml<sup>-1</sup> for 16 h: e, control CHL cells, (♠) or after 60 min in CM at 37° C (○); h, treated with 0.5% deoxycholate for 5 min and lysed immediately (♠) or after 55 min in CM at 37° C (○); h, treated with a 1:500 dilution of antiserum¹0 (○) or with complete medium only (♠).

(3) since Nagle and Belli (unpublished data) observed abrupt DNA degradation in isolated CHL nuclei with intact DNA by adding only ATP, the endonucleases need not be induced but may be constituent to the nucleus; (4) in human lymphocytes which are known to be especially sensitive to X rays. DNA appears to be degraded earlier<sup>12</sup> than in cells considered to be less sensitive, such as LICH or diploid CNS cells of the rabbit6 and dog5, and (5) in all cases, cellular metabolism was required to produce degradation.

It seems clear that similar patterns of degradation are common to various mammalian cells after DNA damage, cell membrane perturbation or general toxicity. The degraded DNA in every case had a modal value of between 106 and 107 daltons. Furthermore, since there is little radioactive label in the first two gradient fractions where mononucleotides and oligonucleotides would sediment, degradation must have resulted from an endonucleolytic incision as opposed to exonucleolytic attack which would produce these small fragments. Our results do not indicate whether the incisional attack on the DNA molecule was at random or rather at particular sites that have structural or functional meaning.

The observation of similar patterns of DNA degradation in cells exposed to widely differing types of trauma suggests that this process is a common mechanism associated with the phenomenon of cell death. As is the case for organisms, cells damaged beyond their recuperative capacity may undergo a distinct, non-reversible process which ends coherent metabolism.

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### matters arising

#### Alpine Fault of New Zealand and Gondwanaland reconstruction

GRIFFITHS<sup>1,2</sup> has proposed two reassemblies of Gondwana fragments in the south-west Pacific, including New Zealand and the surrounding complex of submarine ridges and plateaux. Both recontructions are based essentially on bathymetric data, the salient difference in the later, revised version being a 250 km eastward displacement of Antarctica relative to Australia, to create space for the newly incorporated South Tasman Rise and East Tasman Plateau.

In general terms, Griffiths's reconstructions are feasible, and, as they agree in many respects with my own earlier schematic reconstruction<sup>3</sup> of the region, I do not wish to criticise them except on one important issue. This concerns the nature of the Alpine Fault trace in the reconstituted Gondwana landmass. In his original reconstruction, Griffiths<sup>1</sup> achieves a close fit of south-west Pacific continental and quasi-continental crustal segments by introducing a sharp deflection-through some 70°-in the line supposedly followed by the Alpine Fault. In his revised version<sup>2</sup>, the Alpine Fault trace becomes even more complex and describes a neat parabolic curve with bifurcating and reconverging branches. Neither interpretation is consistent with the known geometry and tectonic behaviour of the Alpine Fault, and, in my opinion, both embody misconceptions

arising from a failure to appreciate that dislocation along the Alpine Fault has been accompanied by substantial deformation of the New Zealand crustal segment.

As I have attempted to demonstrate elsewhere3, the distinctive NE-SW trend of the New Zealand landmass and its congruent regional geological strike both relate to New Zealand's evolution, since late Cretaceous times, along the interface between two moving crustal plates. West of New Zealand, the opening of the Tasman Sea has been attributed4 to late Cretaceous and Palaeocene activity of a NW-SE trans-Tasman spreading axis (perhaps an initial westward prolongation of the still active Pacific-Antarctic Ridge), that brought about a rapid north-eastward migration of the continental and quasi-continental crust of Lord Howe Rise and Norfolk Ridge. To the south and east of New Zealand, extension of the south-western Pacific Basin commenced simultaneously5, and Campbell Plateau and Chatham Risetheir free movement to the north-west obstructed by Lord Howe Rise and Norfolk Ridge-were forced northward at a somewhat slower rate.

These differential movements produced, among other effects, large scale sigmoidal flexuring of the broad belt of Palaeozoic and Mesozoic greywacke formations that had accumulated along the margin of Gondwanaland, in the so-called New Zealand Geosyncline<sup>6</sup>. The development of a complex system of NE-SW major shear fractures, the best known of which is the Alpine Fault, marked the culmination of this flexuring. Within the vicinity of New Zealand, the original course of the peripheral geosyncline can legitimately be supposed to have been more or less linear, and it is essential that, in any reassembly of crustal fragments, compensation be made for the crustal distortion that followed the disruption of Gondwanaland. Figure 1 demonstrates that, in such reconstructions, it is not sufficient merely to postulate reverse displacement of crustal segments by the amount of obvious fault offset. Admittedly, the structural unravelling of intensely deformed areas is not easy, but I would suggest that allowance for crustal distortion, as well as dislocation, in the New Zealand region would simplify and rationalise the form of the Alpine Fault trace in reconstructions such as those of Griffiths.

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DR GRIFFITHS REPLIES-Cullen's main point seems to be that the Alpine Fault is not the only deformation of New Zealand which occurred during seafloor spreading and that it is therefore insufficient simply to reverse the observed 480 km offset in reconstructions. I have, however, always accepted this point; it is not explicitly stated but is quite obvious from the diagrams in my 1971 article (Nature, 234, 203-207). I have suggested (Nature, 235, 83-86; 1972) that the total offset of the New Zealand Geosyncline is some 1,200 km, which is partly taken up by the Alpine Fault, and the rest by 'bending and stretching of the geosynclinal axis". Though detailed structure is not shown, the general concept is quite clear from the figures. I have also referred to the 'Alpine Fault Zone' as distinct from the specific fault (Nature, 249, 336; 1974); it is the former which is indicated on the figure. I have in preparation an enlarged version of my Alpine Fault model, but as it involves over 30 dia-

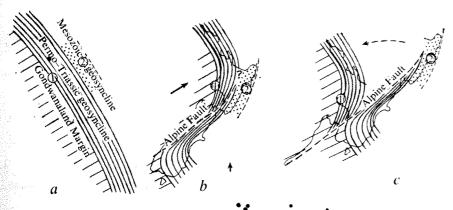


Fig. 1 Schematic reconstruction of the New Zealand region. a, Postulated distribution of linear geosynclinal belts along the eastern margin of Gondwanaland. Numerals represent approximate sites of Egmont (1) and Hawke Bay (2), b, Present day New Zealand structure. Trend and length of arrows indicate direction and rate of Cainozoic crustal movements. c, Spurious Gondwanaland reconstruction (compare Griffiths<sup>1,2</sup>) achieved by simple reversal of the Alpine Fault displacement and realignment of the Permo-Triassic geosynclinal belt. Note that artificial rotation of the Alpine Fault (broken arrow) places Hawke Bay some 500 km north of Egmont, and duplicates a section of the Permo-Triassic geosyncline.

grams, it will be offered for publication elsewhere. In this model, many of the faults in Southland (for example, the Livingstone and Hollyford faults) are related directly to the distortion which Cullen rightly points out has occurred.

In short, I agree with Cullen's observations that further evidence for crustal distortion should be sought, and that the Alpine Fault with its 480 km offset is only part of the whole story. Apart from this, however, his paper seems to offer nothing positive, and indeed fails to recognise that I have always accepted that such distortion must have occurred. If he has a different regional reconstruction, or a detailed model for the deformation of New Zealand, it might help if this was illustrated by accurate diagrams rather than sketches.

University of British Columbia

### Dendrochronology and <sup>13</sup>C content in atmospheric CO<sub>2</sub>

FARMER and Baxter¹ have published δ¹³C values from the wood of tree rings, indicating a decrease over the last 70 yr, which they attribute to the CO₂ increase of the twentieth century. The result, in general, was expected but some implications seem to be questionable, as we can conclude from our results²-⁴.

When studying the decrease of <sup>13</sup>C in atmospheric CO<sub>2</sub>, wood samples have to be selected very carefully. Samples selected from industrial or forest areas do not give reliable information about the average atmospheric composition<sup>5</sup>. The ideal recorder would be a free-standing tree on a small island far out in the ocean. We analysed tree rings of 10 trees (Fig. 1) and recognised large individual variations both in the absolute data and in the trend.

The processing of the samples is also important. It is well known that a network of resin channels permeates the wood tissue and contains soluble organic matter which cannot be correlated to the age of the wood structure. Furthermore, the components which form the wood, cellulose and lignin, have different isotopic compositions. We found a difference between the  $\delta^{13}$ C values of cellulose and lignin prepared from the same wood, of almost 3 ‰. The ratio of lignin to cellulose cannot be expected to remain

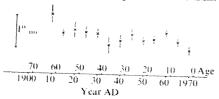


Fig. 1 Average relative <sup>13</sup>C decrease in the wood (cellulose) of tree rings during the past <sup>70</sup> yr obtained from 10 freestanding trees; Quercus robur (4), Q. petraea (1), Pinus maritima (2), Q. lusitanica (2), Platanus acerifolia (1). Each point represents a 5-yr average.

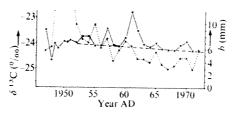


Fig. 2 Platanus acerifolia (Azores), showing correlation between ring width, b (dotted line) and  $\delta^{13}$ C (PDB) values (solid line).

constant over the lifetime of the tree<sup>8</sup>, so that the preparation of one pure component is necessary. The chemical preparation of cellulose<sup>7</sup>, if done carefully, does not introduce artificial isotope fractionation.

Another complication arises from the fact that there are normally considerable variations in the <sup>13</sup>C content even within one single tree ring of the order of 1‰. That demonstrates clearly the necessity for matching wood taken from the same year but from different positions on the tree. Furthermore, a correlation between ring width (climate ?) and <sup>13</sup>C content has been observed (Fig. 2).

Finally, concerning the interpretation of <sup>13</sup>C curves with respect to excess CO<sub>2</sub> in the atmosphere, it has to be taken into account that the exchange rate of isotopic CO<sub>2</sub> molecules between the atmosphere and the oceans is faster than the net flux of CO<sub>2</sub> into the oceans (H.D.F. and L.W., and K. Wagener and H.D.F., unpublished). This means that without any further information it is not possible to draw any direct conclusions from <sup>13</sup>C measurements about the actual CO<sub>2</sub> excess in the atmosphere, even if the fractionation factors are constant.

A detailed presentation of our results will be given elsewhere.

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- <sup>1</sup> Farmer, J. G., and Baxter, M. S., *Nature*, **247**, 273 (1974).
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DRS FARMER AND BAXTER\* REPLY—The data of Freyer and Wiesberg¹ represent a welcome and significant contribution to the preliminary study of  $\delta^{13}$ C trends in recent wood. It is gratifying that their results agree closely with our own²

(Fig. 1) and thereby seem reasonably to negate their criticisms of the different procedures. Although suggestive of a common temporal trend in  $\delta^{13}$ C, their presentation of data as average deviations over 5-yr periods for 10 trees of differing species and locations does not permit inspection of the individual  $\delta^{13}$ C trend for each tree. They thus preclude any precise assessment of discrepancies caused by variations in species or location. Such discrepancies were, for example, noted in our oak and larch data but, of course, are now obscured by the averaging process.

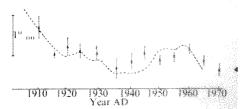


Fig. 1 Comparison of  $\delta^{13}$ C trends in twentieth century wood. Dotted line, mean of data from the same UK oak and larch samples as we reported previously. The points and accompanying errors are those of Freyer and Wiesberg representing the averaged data on 10 selected trees.

In response to specific comments about procedure we would assert that, first, the European larch was indeed selected from a free-standing and exposed situation and yet showed a similar δ13C trend to the forest oak. Second, the dangers of inducing fractionation or contamination during cellulose extraction seem comparable to the possible risks in using whole wood. Third, concerning the ever present difficulty in obtaining a representative biospheric sample, the suggestion of Freyer and Wiesberg. though admirable in intention, could well result in a logistical problem of overwhelming proportions without guaranteeing the comparable representativeness of every sample.

Extreme caution in data interpretation was initially urged by us, and Freyer and Wiesberg are correct to reinforce this view. Nevertheless, it remains undeniable that during the first 20–30 yr of this century, recorded levels of  $\delta^{13}$ C show an unexpected decline which could not have been caused even by complete retention of all the fossil CO<sub>2</sub> introduced into the atmosphere. Furthermore, the CO<sub>2</sub> levels deduced from our  $\delta^{13}$ C values for 1900–1920 agree well with those of Callendar's exhaustive summary<sup>3</sup>, leading to the postulate of an additional biogenic source of CO<sub>2</sub>.

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- <sup>1</sup> Freyer, H. D., and Wiesberg, L., Nature, 252, 757 (1974).
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#### Mononuclear cells killing Cryptococcus

DIAMOND in his report<sup>1</sup> has presented by no means a new discovery; Aronson and Kletter2 have already demonstrated the in vitro killing of extracellular Cryptococcus neoformans by rabbit monocytes even in presence of normal rabbit serum. Monocytes do not necessarily require the presence of specific antibody to kill cryptococcus extracellularly but rather the presence of suitable opsonins which presumably enable the pseudopodia of the phagocytes to adhere firmly to the capsule of this fungus. Rabbit, guinea pig and human sera (but not mouse sera) seem to possess opsonins which are able to induce the phagocytosis of the cryptococci as well as the development of monocyte rings around them which, in turn, can destroy the enclosed parasites.

I feel that Diamond errs when he states that activated macrophages " provided a preferentially favourable medium for intracellular growth of the fungus" and infers that host macrophages may be involved in the dissemination of the disease3. He drew these conclusions from experiments with human monocyte-derived macrophages activated in in vitro conditions with streptokinase-streptodornase and kept in culture for 3-7 d before inoculation of cryptococci. I have observed the extracellular and intracellular killing of cryptococci by murine macrophages (activated either nonspecifically or specifically) in certain conditions: that is, by in vivo-activated adherent cells kept in in vitro culture for only 2 h before the inoculation of cryptococci in the presence of normal rabbit serum4,5; these sera lacked agglutinating capacity. Prolonged in vitro incubation of activated macrophages resulted in a corresponding decrease of their intracellular killing activity.

If phagocyte spreading of the infection in the intact host was involved, as suggested by Diamond, then the administration of drugs like cortisone which are known to promptly depress phagocyte numbers in general should localise this infection. On the contrary, however, it is known that cortisone treatment results in the dissemination of this disease6.7. Diamond's interpretation, that the extracellular killing of cryptococci in his system where 10 µg ml<sup>-1</sup> of cytochalasin B was included to prevent phagocytosis of organisms, was due to antibodydependent killing by monocytes, may not be exactly correct.

Egg-white lysozyme and various cationic proteins from rabbit polymorphonucleocytes are fungicidal for C. neoformans's and the addition of

2-10 µg ml<sup>-1</sup> of cytochalason B facilitates the release of lysosomal enzymes from macrophages by exocytosis<sup>8</sup>. Thus the extracellular killing of cryptococci by enzymes released from monocytes simply as a result of their incubation with cytochalasin B is a possibility which cannot be dismissed by the data Diamond presented.

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DR DIAMOND REPLIES—Sethi seems to have overlooked or misinterpreted the major differences between my studies and those of Aronson and Kletter1. In contrast to their studies, the fungicidal mechanism which I described requires effector cells which are not necessarily monocytes, as well as specific antibody, but no heat-labile The heat-labile opsonins opsonins. which Aronson and Kletter referred to are almost certainly complement components, as detailed in studies of

complement pathways in opsonisation of cryptococci and in the pathogenesis of cryptococcosis2-4 In my study of the possible role of

the role of the classical and alternate

fungicidal activity by non-phagocytic cells, mononuclear cell preparations had 20% or more monocytes mixed with lymphocytes. The effect of heat-labile opsonins was eliminated by heat and the role of other complement components was minimised by the large dilution (1:2,000 or 1:20,000) of antibody. No other serum was present. Unlike the studies of Aronson and • Kletter, • fungicidal activity occurred only in the presence of antibody, even

when effector cells were obtained from four donors who had positive delayed skin tests to cryptococcal antigen.

Sethi's assumption that monocytes are responsible for the fungicidal activity is not warranted. In fact, I

suggested an analogy to antibodydependent cell-mediated lysis of mammalian target cells, which is known to occur when all phagocytic cells have been eliminated from effector cell populations'. In subsequent studies, we used lymphoid cells depleted of phagocytic cells by incubation with carbonyl iron particles and passage through a magnetic field modified from the technique of Lundgren et al.6. Only 43.5% of a crytococcal inoculum survived after incubation for 4 h. This fungicidal activity occurred only in the presence of antibody (R.D.D. and A. C. Allison, unpublished).

The role of the macrophage in immunity to cryptococcosis is another issue entirely, and one which is too complex to be thoroughly discussed here. In any case, corticosteroids have a wide range of effects, and I am surprised that Sethi assumes that depression of the number of phagocytes is the overriding corticosteroid effect on the immune response.

I did not study the mechanism of antibody-dependent killing and certainly did not assume that fungi were killed by monocytes, as Sethi states. Monocytes may well have this killing capacity, and lysosomal enzyme release may well be important. Preliminary studies with my coworkers (R.D.C., C. Cardella, P. Davies, and A. C. Allison, unpublished) have shown, however, that supernatants rich in lysosomal enzymes released from macrophages7 do not kill cryptococci and that cryptococci induce detectable specific lysosomal enzyme release only in the presence of heatlabile opsonins. Anti-cryptococcal antibody augments this release only slightly, if at all.

I regret that Sethi has misunderstood my work, but appreciate the opportunity to rectify the inadvertent omission of reference to the work of Aronson and Kletter, which was called to my attention after my manuscript appeared.

Michael Reese Medical Center, Chicago, Illinois 60616

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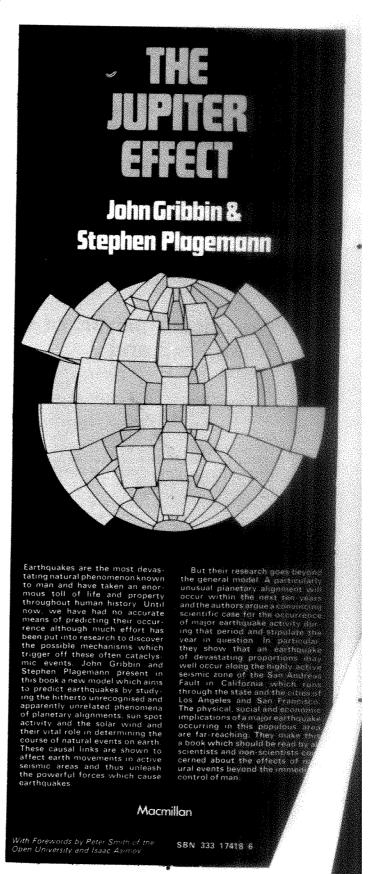
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### reviews

## Triangle of parallel ideas

Leibniz in Paris 1672-1676: His Growth to Mathematical Maturity. By Joseph E. Hofmann. Pp. xi+372. (Cambridge University: London, June 1974.) £8.50.

WHEN Leibniz arrived in Paris in March 1672 as the travelling companion of Melchior Friedrich von Schönborn he knew little of the mathematics of the time. But under the guidance of Huygens he made rapid progress and over the next four years conceived his decisive ideas in mathematics, bringing them to a degree of completeness that allows us to see all his later research as elaboration and development. Leibniz would have liked to remain permanently in Paris but having failed to obtain a post there he reluctantly moved to Hanover in October 1676, where he took charge of the Library. It is a testimony to his irrepressible optimism that despite the uncertainty regarding his future Leibniz succeeded, during his last year in Paris, in attaining his greatest mathematical achievement: the invention of the calculus.

Professor Hofmann's brilliant account of these fruitful years in Paris, originally published as Die Entwichlungsgeschichte Leibnizschen Mathematik während des Aufenthalts in Paris (1672-1676), is now presented in a new version, carefully revised by the author and competently translated by A. Prag and D. T. Whiteside. The story is told with the thoroughness, meticulous documentation and clarity of exposition we have come to expect from the lamented master in the field of Leibnizian mathematics. There are three indexes. The first is a chronological index giving a register of all letters or original publications in contemporary periodicals mentioned in the text or footnotes, locations of manuscripts, meetings of learned societies and a checklist of publications in the periodical literature of the time. This is followed by an index of names and works, and a good subject index.

Leibniz's first mathematical discovery in Paris, concerning the summation of infinite series, illustrates two important characteristics of his work, namely the role played by considerations of logic and his preference for methods rather



Gottfried Wilhelm von Leibniz-did not borrow from Newton

than results. Beginning simply with definitions and the axiom of identity, Leibniz obtained from Grégoire de Saint-Vincent's geometrical progression a general method which enabled him to sum the series of reciprocal triangular numbers, a problem proposed to him by Huygens.

Soon after his arrival in Paris, Leibniz made his first visit to London. Although this was not an unqualified success, it marked the beginning of the exchanges with English mathematicians which led eventually to the priority dispute concerning the invention of the calculus. Having appropriated Sluse's tangent rule. Newton maintained that it was the communication of this rule in his own letter that led Leibniz to develop the ideas of the differential calculus. Leibniz always insisted that he had the rule from Sluse, and an excerpt from Sluse's treatise among his papers confirms the truth of this claim. Hofmann effectively demolishes the idea-unquestioned since the suggestion of Tschirnhaus in 1678-of a link between the ideas of Leibniz and those of Barrow. It was not from

Barrow and Newton but from Huygens, Grégoire de Saint-Vincent, Mercator, Gregory and Sluse that Leibniz received his inspiration. When Leibniz met Tschirnhaus towards the end of November 1675, he already possessed his notation for the infinitesimal calculus. It is clear that Tschirnhaus, who had just visited London, could not have transmitted accurate reports of English mathematical methods to Leibniz (even if he had been told anything in detail) for, as his subsequent correspondence shows, he had not really penetrated to any fundamental level of mathematical understanding.

On the documentary evidence so skilfully presented and analysed in this volume, it is clear that Leibniz had truth on his side when he stressed that, as far as methods were concerned, he got nothing from the English. Hofmann's conclusion seems eminently fair: each of the three rivals (Newton, Leibniz and Gregory) achieved his own method; none borrowed or took over, from either of the others, more than certain incidental details.

E. J. Aiton

#### Phloem transport

Transport of Nutrients in Plants. By A. J. Peel. Pp. 258. (Butterworth: London, April 1974.) £4.80.

THE author's name is a truer guide to the content of the book than the title: transport in the phloem is the subject studied in depth. Discussion of transport in the xylem occupies about a tenth of the book. The symplast system is described, perhaps as an afterthought, in one page of the introduction. Transport across membranes or within the cells is mentioned only briefly. Nevertheless, as a book on phloem transport, this is a most useful work that successfully communicates the nature of scientific investigation and explores a system which is of biological interest to both the academic student and the applied scientist.

Although a self contained book in its own right, the author facilitates further reading by signposting the more relevant recent reviews to augment the information he presents, and by citing some original papers. A glance through the contents rapidly conveys the wide range of background provided. I anticipate that the concepts and approaches discussed will not become rapidly outdated. The book would serve well as an introduction enabling the student to proceed with greater in-

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sight to the proceedings, shortly to be published, of the Phloem Transport Conference just concluded at Banff, Alberta.

The text contains remarkably few factual errors, especially for a first edition. It also presents a fair assessment of the current balance of opinion and of the problems associated with interpreting experimental data in relation to theoretical (or hypothetical) models. The evidence quoted by Peel shows a distinct personal bias, but this prerogative may be allowed as it is relevant work which advances the line of the arguments. Data are quoted with a high degree of reliability throughout the text. In the case where the results presented did not seem to fit-a table on page 30-I found that the error arose only because the data were condensed from two separate experiments in the original reference.

There is a short section which lacks the high standard of clarity shown in the rest of the book, in which Peel calculates how much energy, compared with that available from metabolism, would be required to drive actively a volume flow through sieve tubes containing fibrils. He implies that such a flow would be driven against some overall pressure difference, whereas a metabolically dependent pumping mechanism might well drive a flow against a viscous resistance in the absence of an overall pressure difference. His calculation is, of course, correct but the purpose and the understanding are obscured.

These two criticisms are, however, on very rare exceptions in the text. For the advanced undergraduate, as an introduction to further study, and as a background to applied biology, the book conveys a clear and realistic appraisal of the variety of approaches and of the disagreements and controversies within the field of phloem transport.

David Aikman

Younger fold belts

Mesozoic-Cenozoic Orogenic Belts. Data for Orogenic Studies. Edited and collated by A. M. Spencer. Pp. xvi+809. (Scottish Academic Press: Edinburgh, 1974.) Published for The Geological Society, London. n.p.

This book tells the reader about some 40 segments selected from fold belts formed since Mesozoic times. Dr Spencer writes in his introduction that the volume is not designed for fireside feading, but 1° rather doubt that. It sets out to provide an objective presentation of what is known about each region; as far as possible the treatment is uniform from sector to sector. The result I find is absorbing and the book rather well suited for the evening fireside. With it one can travel along the

margins of the Indian plate from Turkey to the Macquarie Ridge examining 20 sections on the way. And if you fancy the Pacific, there are 20 segments of circum-Pacific structures laid out for your consideration.

About 100 scientists have contributed to the making of this book. As a beginning, a small group worked out a questionnaire which could be applied to each selected segment. It posed over 300 questions relating to such matters as surface shape, deep structure, and time relationships, and was designed to elicit a series of factual answers. The main mass of the book consists of accounts of 46 segments based on the responses of over 80 experts who replied to the questionnaire. Dr Spencer collated and edited these replies. On his shoulders and on Sir Peter Kent, who chaired the group which initiated the project, and who in the later stages provided the resources which brought it to completion, has lain the main burden of producing this volume. Readers can judge for themselves how successful the work has been. Coming to the completed book as a member of the group who started the project I think the result is impressive. Much effort separates the finished work from the initial proposals of the planning committee and I do not feel that membership of that body prevents me from congratulating Dr Spencer and Sir Peter. By their efforts they have transformed the original idea-Mr Brian Harland's, as I recollectinto a book of 800 solidly packed pages.

Geotectonic theorists can now contemplate systematic accounts of segment after segment of the Mesozoic and Tertiary fold belts of the world in comfort by the fireside. More to the point, the book prevides a useful tool for further research. It supplies what was planned at the outset-data for orogenic studies. It shows that the complexities of mountain chains can be treated in a systematic fashion. The amount of data presented varies from region to region, as is to be expected. More serious is the lack of information from the Soviet Union and the western states of America despite vigorous efforts to fill that gap. The Geodynamics Programme includes, however, a similar but more extensive project which may be expected to compensate for those omissions.

Of particular interest is the opportunity this volume provides for comparisons between the make up of young fold belts and structures formed earlier in the history of the Earth. There is a growing body of evidence which suggests that global tectonics have changed with time. Data such as this should help to show whether or not that view is firmly founded. **J. Sutton** 

### Animals' sticking places . . .

Biological Mechanisms of Attachment. By W. Nachtigall. Translated by M. A. Biederman-Thorson. Pp. viii+194+53 plates. (Springer-Verlag: Berlin and New York, 1974.) DM75; \$30.60.

This book is an oddity. As description in hard engineering terms of the ways in which creatures of all kinds attach themselves to their prey, substrate, or each other, it can have little practical value. So it must be read, presumably, for the enjoyment of seeing how nature's engineering parallels that of man.

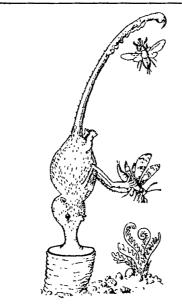
The book is not an exhaustive catalogue (Dr Nachtigall does not describe the flexible limb joints of animals, for example) but it includes devices which hook, grip, clamp, glue, suck or interlock organs or individuals together in permanent or temporary bonds. Any book of this kind is necessarily a list to be dipped into rather than a text to be read from cover to cover, so it is just as well that most of the 712 illustrations are arranged on pages which are opposite their explanations in the text. Where possible, the solutions that human engineers have come up with to meet the same circumstances are described and it is curious how alike many of them are.

Dragonflies, for example, which mate on the wing face problems similar to those of aircraft refuelling in flight, and the mating of the Aedes mosquito (where a perfect alignment of the reproductive tracts must be made and broken in about 14–20 seconds) resembles the 'hard docking' of spacecraft.

Suction seems to be the most widespread attachment mechanism throughout the phyla and about one third of Dr Nachtigall's book is devoted to it. Examples range from the sucking mouthparts of helminth parasites to the suction pads on the feet of the tarsier.

Although arthropods and helminths tend to dominate the book, many old favourites like the pop fastener on the mantle of the squid also appear. The head of the tapeworm, the claw of the arthropod and the Aristotle's lantern of the seaurchin are also rather obvious examples. This indicates a rather subtle drawback of the book: most of the contents, although admirably explained and compared, could probably be guessed by any competent biologist.

There are some surprises—for example, the revelation that no two tissues are ever connected in nature by a third, like a nail or screw—but the merit of the book must remain its value



The fanciful monorhinid, Dulcicauda grisegurell

for directing the biologist to think of the attachment mechanisms of animals in engineering terms rather than as any sort of reference work.

The author makes two curious mistakes. The first (and most serious) is that the text describing the attachment of the sucker organ of the remora does not relate to the series of drawings used to illustrate it—indeed it describes the opposite (and wrong) sequence of events. The second is that the author has placed a charming (but unreal) member of the Monorhina in a section dealing with suction cups when, of course, it uses an adhesive.

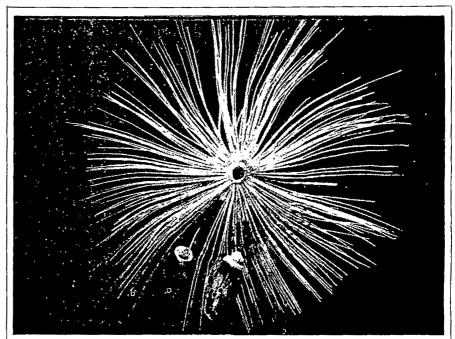
John Wilson

### ... placing sticking plants

Mycologists Handbook: An Introduction to the Principles of Taxonomy and Nomenclature in the Fungi and Lichens. By D. L. Hawkesworth. Pp. 231. (Commonwealth Mycological Institute: Kew, 1974.) £5.50; \$14.30.

Most biologists have only a superficial knowledge and understanding of taxonomic procedures, and those with a real feeling for the subject are few in number. This book goes a long way towards bridging the gap between the many and the few. The author is to be congratulated on producing a detailed, accurate and thoroughly readable book on a subject which is often dismissed as dull and uninteresting.

Although the book is essentially a guide to budding taxonomists it has much to say to others who experiment with, and publish data on, fungi and lichens. The need to identify material correctly, often a difficult task with fungi and lichens, and the need to store specimens in accessible herbaria are particularly emphasised; in the past the frequent failure to do this properly has reduced the value of much published work. Topics covered include the collection and preservation of material; taxonomic ranks; the naming and describing of new taxa; nomenclature; information on authorities and herbaria; and information on the abbreviations of the titles of publications not quoted in the World List of B. W. Ferry Scientific Publications.



A somewhat decorative method of dispersal. The fruit and pappus of the thistle Silybum marianum. From The Natural History of New Zealand: An Ecological Survey. Edited by Gordon R. Williams. Pp. xviii + 434 + 40 plates. (Reed: Wellington, Sydney and London, 1973.) £12.95.

#### **Answers to Monod**

Beyond Chance and Necessity. Edited by John Lewis. Pp. xi+141. (Garnstone: London, 1974.) £2.95.

When Jacques Monod, in Chance and Necessity, classed Christians and Marxists, along with witch doctors, as "animists" who attribute a will to nature, a reply was to be expected. These essays, published as part of the Teilhard Study Library, are part of that reply; Beyond Chance and Necessity brings together a number of people who have little in common other than their disagreement with Monod.

Monod argued that our present cultural confusion arises because our ethical beliefs are based on various pictures of the world, in particular the Christian myth, which ascribes a purpose to the Universe and a special role for man in that purpose, whereas our economic existence depends on the scientific world picture, which does not admit explanations in terms of purposes or final causes, and which sees man as the accidental consequence of natural selection acting on random mutation. He urges us to recognise the distinction between 'objective' statements proper to science, and statements of value which cannot be derived from science. The 'principle of objectivity'-the avoidance of teleological explanation in science—he sees as itself a moral commitment, necessary for the practice of science and, therefore, not derivable from it.

Mary Warnock accuses Monod of arguing in a circle. If science is based on the assumption that there are no purposes in the Universe, one cannot then deduce from science that there is no purpose, and no special role for man. Monod's picture of the world is, in fact, quite consistent with God as a 'first cause', who created a world capable of generating life, and who even retains an interest in that world. This argument is taken further from a Christian standpoint by Arthur Peacocke. He argues, by analogy with statistical mechanics, that the randomness of mutation does not mean that there are no laws of evolution; a point made by other contributors, and one I would accept. For a Christian, however, God as a first cause who designed the laws of nature and the initial conditions to hold the potentiality of life and who then let the Universe run, is not enough. Peacocke is critical of the concept of a "God of the gaps", invoked to explain those phenomena which science cannot, because that canelead only to a dwindling role for God. Instead he sees God as imminent in all natural processes, working through the laws of nature and not by suspending them.

My difficulty here is not so much that I disagree with him as that I cannot see what he can possibly mean. If God is simply another name for the laws of nature, then not only is there no need for that hypothesis, there is no need for the term itself.

The defence of Marx is taken up by John Lewis. It is perfectly possible to be a good philosopher without knowing any biology; unhappily, Dr Lewis writes as if he understood biology when it is quite clear that he does not. That gives such an air of insincerity to his essay that I found it impossible to pay serious attention to it. That is a pity, because I think he is right in arguing that Marx and Engles were not animists in Monod's sense. They saw 'dialectical laws' not as a mind or purpose in nature, but as observable regularities in the behaviour of natural systems.

Joseph Needham also comes to the defence of Marxism. He quotes with approval the antimechanist views expressed by Soviet Marxist scientists in the early 1930s. That will not quite do. A few years later, Marxist philosophers, arguing that the gene was an undialectical entity because it controls development without being influenced by it, supported the destruction of biology in the Soviet Union; at the same time, men who believed that the cell is a machine (not, it is true, a clock—more a tape-recorder) were revolutionising biology.

The most interesting essays in the book are written from within evolution theory and molecular biology, by C. H. Waddington and Robin Monro. Waddington was provoked into contributing by a report that Monod has accused him of Lysenkoism. I do not know whether the report is true, but I am glad of the result; if a man is accused of Lysenkoism it does concentrate the mind wonderfully. Waddington gives an unambiguous account of the significance of genetic assimilation. Monro criticises Monod's excessive reliance on molecular concepts in biology. He first argues that the 'central dogma'-that information cannot pass from protein to nucleic acids-is not necessary for the truth of Weismannism. An 'acquired character' is not usually reflected in a changed amino acid sequence in protein, so even if the central dogma were false, most acquired characters could not be transmitted. Monro goes on to argue that the dogma is not sufficient either, because some kind of germinal selection may produce Lamarckian effects (the same idea is worked out in more detail by Waddington).

The main difficulty in assessing this controversy is that at least two separate issues are being confused. The first is the relationship between science and

ethics. There, I think Monod was right in saying that the scientific world picture has been built by abjuring final causes. I have little sympathy with those of his critics who dislike mechanistic theories in science because they lack moral uplift. But there is second issue, concerning the strategy of scientific research; there, I think Monod's critics have a case. I do not think it will prove possible to explain biology in molecular terms in quite the way Monod hopes. For example, he writes "personally I am convinced that in the end only the shape-recognising and stereospecific binding properties of proteins will provide the key to these phenomena" (that is of development). My own guess is that it will no more be possible to understand the shapes of embryos in terms of the properties of proteins than it is possible to understand the shapes of the waves generated in Jabotinski's reaction in terms of the shapes of the constituent molecules. Of course, theories of development will be dependent on and consistent with molecular biology but they will not be discovered by thinking only at the molecular level.

This guess may be wrong. But then, I wish I was as sure of anything as Monod is of everything. How a man can write "This central concept of modern biology is no longer one among other possible or even conceivable hypothesis, the only one compatible with observed and tested fact. And nothing warrants the supposition (or the hope) that conceptions about this should, or ever could be revised", and claim to be a Popperian, I do not know.

John Maynard Smith

### Critical look at the LMFBR

The Liquid Metal Fast Breeder Reactor. By Thomas B. Cochran. Pp. xiv+271. (Johns Hopkins University: Baltimore and London, 1974.) \$6.95.

I CANNOT do better than quote from the preface:

"In this monograph Thomas Cochran takes a critical look at the economic and environmental arguments which have been made in favor of an early introduction of the liquid metal fast breeder reactor (LMFBR) as a central component of the United States electrical energy system."

Taking a number of published United States (US) reports, particularly the cost/benefit analyses put out by the US Atomic Energy Commission in 1970 and 1972, he considers in reasonable detail the choice of discount rate, the assessment of generating costs, the validity of currently assumed perform-

ance parameters, fuel cycle costs, and the arguments relating to the supply and demand of uranium.

Cochran may be right in parts of his criticism, but recent history has shown the need for frequent revision both of the ground rules in policy analyses and of the assumptions that form the background to them. In keeping with his pessimistic view of the LMFBR he suggests the continued use of thermal reactors and the stock-piling of the depleted uranium for future use—maybe not with LMFBRs. He advocates further investigation of alternative energy sources, without offering much hope of better success in any other direction.

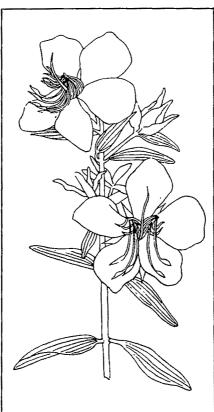
That part of his submission which challenges the proposal to build many fast reactors between 1986 and 2000 is perhaps less important in terms of today's decision than his critical comparison between the LMFBR and the light-water reactor. Neither in the US nor the UK do we yet know what new information and experience will be available by 1986, and the present need is to ensure a capability of energy production in the latter part of this century. To that end the UK, France, Germany, Italy, Japan and the Soviet Union are actively developing the LMFBR—a system with which Europe has considerably more experience than the United States.

Many of the problems Cochran raises are common to any programme of nuclear power, such as the long-term storage of radioactive waste and the processing and transport of fuel. These become more acute with the rate of increase of the total power programme and are little affected by the choice of systems.

Mr Cochran has made an excellent attempt to analyse the safety of the fast reactor in simple terms. I wish to comment only on a few exceptions: for example, the difference in neutron lifetime is barely relevant to most accident conditions as the rate of change of temperature is dictated by the doppler coefficient, that is, controlled by the thermal inertia of the fuel. A prompt, critical situation cannot be controlled by mechanical systems, neither in thermal nor in fast reactors. The importance of the sodium void coefficient arises only if coolant is ejected from a substantial number of fuel channels. The way in which an accident might develop will be different in different reactor systems but the end effect of the potential thermal interaction of hot fuel and coolant can he violent in water reactors as well as in the LMFBR and the available energy is not markedly different.

There is a growing recognition that there is some uncertainty and consequent risk in many industrial activities, not only in the development of nuclear power, and it is obviously important to be aware of these risks in order to minimise them. This monograph should encourage further critical reviews of the US programme and the part to be played by fast reactors in a future energy economy.

F. R. Farmer



Dissotis speciosa. From Upland Kenya Wild Flowers. By A. D. Q. Agnew. Pp. ix +827. (Oxford University Press: London, November 1974.) £7.75.

### Happy union in the feldspar family

Feldspar Minerals. Vol. 1: Crystal Structure and Physical Properties. Pp. xx+627. Vol. 2: Chemical and Textural Properties. Pp. xi+690. By J. V. Smith. (Springer-Verlag: Berlin and New York, 1974.) Vol. 1: DM98.30; \$40.10. Vol. 2: DM103.50; \$42.30.

THESE splendid volumes on the most abundant, ubiquitous and internally complex minerals in the Earth's crust set a new standard for advanced mineralogical texts. They are extraordinarily comprehensive and the reader must feel a sense of wonder at the author's capacity to organise so vast a body of literature into a coherent and remarkably readable whole. The hundreds of references are very up to date (1973) and much use is made of preprints and theses. The author's aim is avowedly pedagogic, to use the feld-

spars to show how physical and chemical principles can be combined with geological observations to contribute to our understanding of mineral genesis. The books therefore contain several crisp but useful sections outlining most of the many physical and analytical techniques applicable to crystalline materials in general, with many references to basic papers which develop physicochemical principles. At the other extreme, volume 2 covers at length a multitude of petrological observations on feldspar twinning and integrowths. In their marriage of crystallographic sophistication with grass-roots geological observation (a union sadly missing in much feldspar research) the books set an example applicable across the whole mineralogical field.

The volumes are so comprehensive that feldspar specialists are likely to find their own field dealt with at length, with a most valuable review of all the relevant literature; for more general readers certain sections stand out. In volume 1 the structural architecture of the feldspars is developed in great detail, beginning with discussion of models of bonding. The unsolved intricacies of the one or two-step ordering problem in K-feldspars are discussed and later related to the difficulties of classification of the K-feldspars. These sections as nomenclature are required reading for petrographers, who will also welcome the compilation of X ray and optical determinative techniques. Electron-optical methods will also find increasing use as a petrographer's tool and photographs of many fascinating (and sometimes enigmatic) textures are reproduced clearly.

Volume 2 begins with an outline of analytical techniques followed by a lengthy description of chemical substitution. Mechanisms of growth, development of chemical zoning, morphology and all the many feldspar twins are described, with structural explanations and a widely applicable discussion of twinning mechanisms. Similarly, intergrowths of feldspar with feldspar and with a host of other minerals are explained in terms of mechanisms of exsolution and replacement. Volume 3, dealing with phase equilibria and petrogenesis (promised for 1976) must be awaited eagerly by all mineralogists and petrologists. These lavishly produced, totally comprehensive volumes bring out, through the author's obvious enthusiasm, all the fascination there is in using modern techniques to explain mineral variation and it is to be hoped that they will be treated not just as invaluable works of reference, but also as a guide to all that is possible in modern mineralogical work.

Ian Parsons

#### announcements

#### **Appointments**

Francis Joseph McQuillin has been appointed to a personal chair of organic chemistry at the University Newcastle-upon-Tyne.

George Albert Swan has been appointed to a personal chair of organic chemistry at the University Newcastle-upon-Tyne.

#### Awards

J. Baddiley has been awarded the Davy Medal by the Royal Society for his work in the field of chemistry.

W. G. Richards has been awarded the Butterworths Scientific Fellowship for Authors for 1975.

#### **International meetings**

February 3-7, 1975 National Symposium on Forensic Sciences, Perth (Mr V. J. McLinden, Secretary, Australian Forensic Society, Government Chemical Laboratories, 30 Plain Street, Perth, Western Australia 6000).

February 4, Entry and Distribution of Pollutants in Groundwater and Rivers, London (The Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London SW1X 8PS).

February 6, The Role of Toxicology in the Development of a Drug, London (Dr J. F. Cavalla, John Wyeth & Brother, Ltd., Huntercombe Lane South, Taplow, Maidenhead, Berk-

February 17-18, Glacial Till, Ottawa (M. K. Ward, Executive Secretary, Conference on Glacial Tills, c/o National Research Council of Canada, Ottawa, Canada K1A 0R6).

February 24-27, 4th Conference on Experimental Medicine and Surgery in Primates, Jerusalem (The Secretariat, P.O.B. 16271, Tel Aviv, Israel).

March 3-7, 26th Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Cleveland, Ohio (Mr S. David Cifrulak, Calgon Corporation, Pittsburgh Activated Carbon, PO Box 1346, Pittsburgh, Pennsylvania 15230.

March 4, Aspects of Fungicidal Selectivity, London (The Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London SW1X 8PS).

March 10-15, Dahlem Workshop on the Nature of Seawater, Berlin (Dahlem Konferenzen, 1 Berlin 33, Delbrückstrasse 4c, Germany).

March 13-14, Symposium on Infections of the Foetus and Newborn Infant, New York (Dr Saul Krugman, New York University Medical Center, School of Medicine, 550 First Avenue, New York, N.Y. 10016).

March 13-15, Energy Transducing Membranes: Structure, Function and Reconstitution, Buenos Aires (Dr Alberto A. Boveris, Universidad de Buenos Aires, Facultad de Medicina, Instituto de Quimica Biologica, Pàraguay 2155, Buenos Aires, Argentina).

March 17-21, Biological Microanalysis, Paris, (Dr Patrick Echlin, Biological Microprobe Laboratory, Department of Zoology, Downing Street, Cambridge CB2 3EJ, UK; or Professor Pierre Galle, Faculté de Médecine de Créteil, 6 rue du Général-Sarrail, 94010 Créteil, France).

March 24-25, Zmuda Memorial Conference on Geomagnetic Field Models, Colorado Springs (American Geophysical Union, 1707 L Street, NW, Washington DC 20036).

March 24-26, Europhysics Conference on Nuclear Interactions at Medium and Low Energies, Harwell (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

March 26, The Structure and Origin of Comets, York (Professor M. M. Woolfson, Department of Physics, University of York, Heslington, York YO1 5DD).

March 31-April 3, 5th International Symposium on Flavins and Flavoproteins, San Francisco (Dr Thomas P. Singer, Molecular Biology Division, Veterans Administration Hospital, 4150 Clement Street, San Francisco, California 94121.

#### Reports and publications

#### Great Britain

Annual Report of the Scottish Marine Biological Association for the year ending March, 31, 1974. Pp. 69. (Oban, Argyll: Scottish Marine Biological Association, 1974.) 75p. [710]
Keyword Index of Guides to the Serial Literature. By A. G. Myatt. Pp. 35. (Boston Spa, Wetherby: The British Library, Lending Division, 1974.) £1. [810]
Science Research Council. Greenwich Time Report. Royal Greenwich Observatory Time and Latitude Service, 1973 July—December. Pp. 369–396. (London: Science Research Council, 1974.) [1010]
Imperial College of Science and Technology, (University of London). Safety Precautions in the Use of Practice Against Radiation Hazards. Sixth edition. Pp. 72. Precautions Against Biological Hazards. Pp. 119. Safety in Chemical Laboratories and in the Use of Chemicals. Third edition. Pp. 46. (London: Imperial College of Science and Technology, 1974.) [1010]
The Hostile Environment of Man (Report of a Symposium held on May 1, 1971, at the Use Inversity of

The Hostile Environment of Man (Report of a Symposium held on May 1, 1971, at the University of Leeds.) (The Journal of the Royal College of General Practitioners, Supplement No. 1, Vol. 24, 1974.) Pp. 46. (London: The Royal College of General Practitioners, 1974.) £1.25. [1010]

Proceedings of the Royal Irish Academy, Vol. 74, Section A, No. 13: Power Mappings and Group Morphisms. By D. MacHale. Pp. 91–94. 14p. Vol. 74, Section B, No. 16: The Ecology of Collembola in Irish Blanket Bogs. By R. E. Blackith. Pp. 203–226. 38p. Vol. 74, Section B, No. 17: The Stratigraphy of the Carboniferous Rocks of Hook Head, Co. Wexford. Pp. 227–244. 33p. (Dublin: Royal Irish Academy, 1974.)

#### Other countries

United States Department of the Interior: Geological Survey. Professional Paper 829: Geology and Mineral Deposits of the Poncha Springs SE Quadrangle, Chaffee County, Colorado. By Ralph E. Van Alstine. Pp. iii + 19 + plate I. (Washington, DC: Government Printing Office, 1974.) \$1.95.

Office, 1974.) \$1.95. [309]

Smithsonian Contributions to Botany, No. 14:
Commercial Timbers of West Africa. By Edward S. Ayensu and Albert Bentum. Pp. iii + 69. \$1.65. Smithsonian Contributions to Anthropology, No. 18:
Reconstruction of Demographic Profiles from Ossuary Skeletal Samples—a Case Study from the Tidewater Potomac. By Douglas H. Ubelaker. Pp. xi + 79. \$2.25. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.)

Transactions of the American Philosophical Society.
New Series, Vol. 64, Part 4: Mappae Clavicula—a
Little Key to the World of Medieval Techniques. By
Cyril Stanley Smith and John G. Hawthorne. Pp. 128.
(Philadelphia: The American Philosophical Society,
1974.) \$7.

East African Community. East African Virus Research Institute Report for 1972. (No. 22). Pp. ii + 56. (Entebbe, Uganda: East African Virus Research Institute, 1972. Shs. 12.

Institute, 1972. Shs. 12. [210]
Theoretische Aspekte der Menschwerdung. Von B. Rensch und J. L. Franzen. (Aufsatze und Reden der Senckenbergischen Naturforsheenden Gesellschaft.) Pp. 59. (Frankfurt am Main: Verlag Waldemar Kramer, 1974.) DM. 20.

1974.) DM. 20. [210 Lectures in Plasma Physics: The Magnetohydrodynamic Approach to the Problem of Plasma Confinement in Closed Magnetic Configurations. By C. Mercier, in collaboration with H. Luc. (Research Group of the Association EURATOM-CEA, CEN/Fontenay-aux-Roses (France). Pp. (Luxembourg: Commission of the European Communities, 1974.) [310 Professional Profile, Vol. 1, No. 1, 1974. Pp. 1-36, (Eindhoven, The Netherlands: NV Phillips Gloeilampenfabricken, 1974.) [410 Canada, Penaturent of Energy Mines and Research

(Eindhoven, The Netherlands: NV Philips Gloeilampenfabrieken, 1974.)

Canada: Department of Energy, Mines and Resources. Economic Geology Report No. 29: Niobium (Columbium) and Tantalum in Canada. By K. R. Dawson. Pp. ix + 157, \$4.50, Paper 74-22: Gravity and Magnetic Natural Resource Maps (1972) Offshore Eastern Canada—Philosophy and Technique in Preparation by Computer. By Richard T. Haworth. Pp. 15, \$3. Paper 74-24: Calliergon aftonianum Steere in Late Tertiary and Pleistocene Deposits of Canada. By M. Kuc. Pp. 6. (2 plates), \$2. Paper 74-32: Geochemical Studies in the Surficial Environment of the Beaverlodge Area, Saskatchewan. By Willy Dyck. Pp. 30, \$3. Paper 74-45: Elemental Associations of Mineral Deposits and Indicator Elements of Interest in Geochemical Prospecting (Revised). By R. W. Boyle. Pp. 40, \$2. Paper 74-52: Field Data Acquisition Methods for Applied Geochemical Surveys at the Geological Survey of Canada. By Robert G. Garrett. Pp. 36, \$3. (Ottawa: Information Canada, 1974.) [410]

Environment Canada: Fisheries and Marine Service.

Pp. 36. S3. (Ottawa: Information Canada, 1974.) [410 Environment Canada: Fisheries and Marine Service. Technical Report No. 466: An Attempt to Overwinter Salmonids at Prevailing Seawater Temperatures in New Brunswick. By R. L. Saunders, B. C. Muise and E. B. Henderson. Pp. 14. Technical Report No. 480: User's Manual for Pisces—a General Fish Population Simulator and Fisheries Program. By A. V. Tyler. Pp. 30. (St. Andrews, N.B.: Research and Development Directorate, Biological Station, 1974.) [710 Australia: Commonwealth Scientific and Industrial Research Capanization. Annual Report of the Minerals Research Laboratories, 1973/1974. Pp. 71. (Melbourne: CSIRO, 1974.) [710 Practical Building of Methane Power Plants for

CSIRO, 1974.)

Practical Building of Methane Power Plants for Rural Energy Independence. By L. John Fry. Edited by D. Anthony Knox. Pp. 96. (Santa Barbara, California: Standard Printing, 1974.)

Journal of Environmental Economics and Management, Vol. 1, Nl. 1, May 1974. Pp. 1–88. Edited by Allen V. Kneese and Ralph C. d'Arge, Subscription: Vol. 1 (4 issues), 1974, \$30. (New York and London: Academic Press, 1974.)

Australia: Commonwealth Scientific and Ledward.

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Plant Industry, 1973. Pp. 171. (Canberra: CSIRO, 1974.)

United States Department of the Interior: Geo-logical Survey. Professional Paper 830: Zeolites and Associated Authigenic Silicate Minerals in Tuffaceous Rocks of the Bog Sandy Formation, Mohave County, Arizona. By Richard A. Sheppard and Arthur J. Gude, 3rd. Pp. iv 1 36. (Washington, DC: Govern-ment Printing Office, 1973.) \$1.05.

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New Zealand. Report of the Department of Scientific and Industrial Research for the vear ended! Martin 1974. (G21). Pp. 68. (Wellington: Grovenment Printer 1974.) 45c. [810]
United States Department of the Interior: Geological Survey. Professional Paper 772. [Gonl. Bearing Gravel of the Ancestral Yuba River, Sieria, Nevada, Canifornia, By Warren E. Yeend. Pp. iv 44. 2. [1346.] (Washington, DC: Government Printing Office, 1974.) 52.70.

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Classified Advertisements

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#### APPOINTMENTS VACANT

#### "METALS ABSTRACTS"

The international abstracting service for metallurgy offers permanent positions as SENIOR EDITORIAL ASSISTANTS. The work consists of editing and checking abstracts for publication. A science degree, preferably in metallurgy, physics, or chemistry, is necessary and a working knowledge of a foreign language would be an advantage.

Applications, stating age, education, qualifications, and experience, to Dr T. Graff, Metals Abstracts, The Metals Society, I Carlton House Terrace, London SWIY 5DB.

#### DEAN OF THE FACULTY OF SCIENCE

The newly-established Faculty of Science of the University of Regina requires a dean to consolidate programs and provide leadership. The University is seeking a person with an established reputation in research and with successful administrative experience. The Faculty contains six departments and 70 permanent faculty members. The Faculty offers three and four year undergraduate programs and graduate programs to the Ph.D. level.

Please apply to Mr. D. T. Lowery, University Secretary, University of Regina, Regina, Saskat-chewan, Canada, S4S 0A2. (2195)

#### DEPARTMENT OF PHYSICS UNIVERSITY OF ALBERTA ELECTRONMICROSCOPIST

Applications are invited for an Assistant Professor in the area of Electronmicroscopist. Exceptional candidates in other areas of condensed state physics will be considered. The condensed state group at the University of Alberta consists of eight members in a Physics Department of forty-two faculty members. faculty members.

The effective date of appointment is April 1, 175. The closing date for applications is February 1, 1975.

Send vitae, list of publications, and names of three referees to: Dr J. T. Sample Chairman

Department of Physics
University of Alberta
Edmonton, Alberta, T6G 2E1 (X1911)

#### UNIVERSITY OF ALBERTA Edmonton, Alberta, Canada ASSISTANT PROFESSOR PHYSIOLOGY

Salary negotiable, M.D. and/or Ph.D. Strong research interests or interest and teaching ability essential. Apply with curriculum vitae and names of three referees by January 24, 1975.

Applications to: Dr M. Schachter, Professor and Chairman, Dept. of Physiology, University of Alberta, Edmonton, Alberta, Canada. (2237)

### Laboratory Manager for Evans Biologicals Limited from £2,700

We require a manager to take charge of a laboratory which responsible for the preparation and growth testing of tissue cultur for production, research and test programmes. In addition, at a lat stage, responsibility for the control of the safety testing of vir vaccines will be involved.

Candidates, who must have relevant experience as a laborato manager, should have a degree or H.N.C. in a biological subject or ha an I.M.L.T. qualification.

We are one of the country's leading manufacturers of human a veterinary vaccines and are located at Speke near Liverpool, with easy travelling distance of pleasant residential areas in Cheshire, t Wirral and on the Lancashire coast.

Starting salary will be from £2,700 according to qualifications a experience. In addition the conditions of employment include 4 wee which we are a member and assistance with re-location expen where appropriate.

Please write with brief career details, quoting reference U.53, t



Dr M. C. Cook, Management Staff Adviser, Evans Medical Limited, Speke, Liverpool L24 9JD.

(2257

Ministry of Agriculture, Fisheries and Food Torry Research Station, Aberdeen

### Microbiologist

Work on National Collection of Industrial Bacteria Ma groups of bacteria and bacteriophages Operate identification vice Research into methods of identification, taxonom preservation of bacteria.

Ist/2nd hons degree or equivalent in Microbiology [, Age unde Appointment as Scientific Officer (over £1800 to £2900) . Ref: SB

■ For application form see below.

Imperial War Museum Film Archive, Hayes, Middlesex

■ To research into preservation of film archive ■ Undertak ical and instrumental testing of cellulose nitrate cine-film in development of colour preservation techniques and protec

[] Application forms (for return by 17 January 1975), from Civ Commission, Alencon Link, Basingstoke, Hants RG21 1]B, Basingstoke 29222 ext. 500 (or, for 24 hour answering service 01-839 1992).



### SIMON FRASER UNIVERSITY DEPARTMENT OF GEOGRAPHY

Burnaby, B.C., Canada

Position: Chairman, Department of Geography, Senior academic preferred, active scholar with strong interest in teaching, Canadian experience desirable. To Chair department for a renewable term of 2 to 5 years.

Rank and salary negotiable, Appointment on or before September 1, 1975.

Applications, with curriculum vitae, to be sent to:

Dean W. A. S. Smith Faculty of Arts Simon Fraser University Burnaby, B.C. V5A 1S6 Canada.

Deadline for applications is January 15, 1975 but applicants are encouraged to apply as early as possible.

#### UNIVERSITY OF WESTERN ONTARIO DEPARTMENT OF ZOOLOGY ASSISTANT PROFESSORS

Applications are invited for the positions of: VERTEBRATE POPULATION BIOLOGIST familiar with quantitative analysis and specialising in population dynamics, ecological genetics, behaviour or reproduction; and ECOLOGIST specialising in aquatic populations or ecosystems. Both positions are three year probationary appointments starting in July or September 1975. Starting salary of at least \$13,025. Applicants should have demonstrated ability to develop active independent research programmes and be familiar with modern teaching methods.

Send curriculum vitae (include telephone number) and three letters of reference by February 1, 1975

Or M. Locke, Chairman, Department of Zoology, University of Western Ontario, London, Canada N6A 3K7.

#### UNIVERSITY OF BRISTOL ION THRUSTERS FOR SPACE PROPULSION

Applications are invited for the post of Research Assistant at postdoctoral or equivalent level in the Department of Aeronautical Engineering, to start a new research project, sponsored by the S.R.C., on the use of xenon for an ion thruster.

The work will involve the design and construction of a suitable test facility, and the investigation of the performance of an ion thruster using xenon gas as a propellant. The thruster will be provided by the Space Department of R.A.E. Farnborough.

Applicants should have a good degree in Physics or Engineering; preference will be given to applicants with appropriate research or industrial experience, particularly in plasma physics. The starting salary will be up to £2,412 per annum, plus Threshold Agreement supplements. The appointment would be expected to continue for three years.

Applications, giving details of qualifications and potgraduate experience and the names of two referees, should be sent to Professor L. F. Crabtree, Queen's Building, Bristol BS8 1TR. (2252)

### OXFORD UNIVERSITY PHYSICAL CHEMISTRY LABORATORY AND ST JOHN'S COLLEGE

AND ST JOHN'S COLLEGE

Applications are invited for a joint appointment to a Departmental Demonstratorship in Physical Chemistry and a Fellowship by Special Election at St John's College. The appointment will be for a limited term, which will not exceed five years, and at a joint salary on the scale of a University Lecturer and Official Fellow. It is hoped to make an appointment starting in either April or October 1975. For further particulars of the Demonstratorship please write before February 1, 1975, to Professor J. S. Rowlinson, Physical Chemistry Laboratory, South Parks Road. Oxford OX1 3QZ. England, who will also forward particulars of the College Fellowship. (2253)

#### THE UNIVERSITY OF LANCASTER

DEPARTMENT OF ENVIRONMENTAL SCIENCES

### LECTURESHIP IN GEOPHYSICS

Applications are invited for the above post tenable from October 1, 1975. The successful candidate will be required to teach undergraduate courses in geophysics and to assist with teaching in related subjects.

The main departmental research interests in solid earth geophysics at present are concerned with Crustal and Upper Manute Studies using deep magnetic soundings, with facilities for complementary gravity and seismic studies and for magnetic survey. Preference will be given to a candidate with research interests in deep magnetic sounding or related fields.

Further particulars may be obtained (quoting reference L.857/C) from the Establishment Officer. University House, Lancaster LA1 4YW, to whom applications (five copies), naming three referees, should be sent not later than February 28, 1975. (2248)

#### CELLULAR IMMUNOLOGIST

Post- or pre-doctoral, with experience of culture methods, to join a team investigating lymphocyte and phagocyte function in relation to immunodeficiency and grafting, tumour immunotherapy and rheumatoid diseases.

Salary, Whiteley A scales, up to £3,144 p.a.
Applications by January 6 1975 to Professor
J. R. Hobbs, Westminster Medical School, 17
Page Street, SW1P 2AR. (2255)

Applications are invited for a

## lectureship in Astrophysics

at the Astronomical Institute.

Duties will include research and teaching activities (graduate and undergraduate level).

Applicants should have a good knowledge of astrophysical observing techniques - especially stellar spectroscopical - and a thorough insight of the physical processes bearing on stellar evolution. The present research interests are in hydrodynamical processes, nucleosynthesis, and high energy electromagnetic processes (X-ray and ultraviolet radiation of stars and astrophysical plasma's). Salary in accordance with background and experience.

Send curriculum vitae, names of at least two referees, and present research interests, quoting nr. 185 AX to: prof. dr. E. P. J. van den Heuvel, Astronomical Institute, Roetersstraat 15, Amsterdam, The Netherlands.

Applications should be sent not later than January 15, 1975.



Universiteit van Amsterdam

#### OXFORD POLYTECHNIC

Applications are invited for the post of

#### SENIOR LECTURER/ LECTURER II IN GEOLOGY

to take charge of teaching, and initiate research in Geochemistry.

Further details and application

Deputy Director, Oxford Polytechnic, Oxford OX3 0BP. (2243)

#### M.R.C. CLINICAL RESEA'RCH CENTRE

(Northwick Park Hospital) Watford Road, Harrow, Middlesex HA1 3UJ TUMOUR IMMUNOLOGY

#### JUNIOR TECHNICAL OFFICER

to work in the Division of Surgical Sciences in research in cellular immunology, with particular emphasis on tumour immunology. This involves some small animal work and a wide variety of in vitro techniques. Salary within the range £1,875 to £2,718 plus £228 threshold. Candidates with degree, H.N.C. or equivalent should apply to Mrs J. Tucker-Bull for application forms.

Please quote ref. 128/2/4302.

### Young Morphologist

YOUNG MORPHOLOGIST required immediately for scientific staff of research unit working in development neurobiology field. Unit now at Carshalton, but a move to WC1 area of London is

Applicants should have at least 3 years' postgraduate experience. The studies will be concerned with investigations on the developing Central Nervous System with light and electron microscopy, and will also involve co-operative studies with biochemists and neuroendocrinologists working in the Unit.

Appointment will be for 3 years; salary in the range £2,019 to £3,636; F.S.S.U. Superannuation.

Apply to Dr R. Balázs, Neuropsychiatry Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey. (Tel. 01-643 8000).



#### UNIVERSITY OF QUEENSLAND Australia

#### SENIOR LECTURER/LECTURER IN BIOMETEOROLOGY

(Environmental Biophysics)

The Department of Botany invites applications from biometeorologists to undertake teaching and research in the field of energy dynamics of plant communities in co-operation with plant ecologists and physiologists. An understanding of hydrology and systems analysis would be desirable. January 3, 1975.

#### SENIOR TUTOR IN BIOCHEMISTRY

Applicants should have as minimum qualifica-tions an honours science degree, but preferably should have a higher degree. Applicants should indicate their research interests. They will be re-quired to do some lecturing and to direct some laboratory classes. January 10, 1975.

GENERAL CONDITIONS: Salary (under review)— Senior Lecturer \$A12,643 to \$A14,724; Lecturer \$A9,002 to \$A12,352; Senior Tutor \$A7,545 to \$A9,002 per annum.

OTHE BENEFITS: Lecturer and above—Super-annuation similar to F.S.S.U., housing assistance, reaveiling and removal expenses.

Senior Tutor—Superannuation similar to F.S.S.U., housing assistance, travelling and removal expenses.

Additional information and application forms are obtainable from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WCIH 0PF. (2235)

CAMBRIDGE AREA HEALTH AUTHORITY (TEACHING)

CAMBRIDGE HEALTH DISTRICT (TEACHING)

ADDENBROOKE'S HOSPITAL SCIENCE GRADUATE

MEDICAL TECHNICIAN

required for a research project involving cellular DNA Assessment. This post, sited in the Department of Haematology, is subject to Whiteley Council conditions of service.

Further information may be ob-

Further information may be obtained from Mr D. W. Finch, Haemotology Department. Application Forms from the District Personnel Officer. Addenbrookes Hospital, Hills Road Cambridge. Road, Cambridge. (2259)



Pharmaceuticals, Toiletries Hospital Supplies

### **Physiological** Chemistry

The special Projects Group in the Development Division of Nicholas, based at Slough, undertakes fundamental studies of a typical chemical nature in a number of fields of pharmaceutical interest.

We are currently seeking a person with a knowledge of physiology, preferably with M.Sc. or equivalent post graduate experience, to strengthen the group's expertise in the subject.

The position will provide the opportunity to work in a wide range of subjects with a high degree of autonomy. The successful applicant should be prepared to adopt an interdisciplinary approach to problems, to take an active part in problem definition, and should preferably have some knowledge of discussion and membrane process.

A competitive salary will be paid and the company offers above average conditions of employment. Please write or telephone for an application form to: Ron Klerks, Personnel Officer, Nicholas Laboratories Limited, 225 Bath Road, Slough, Berks. Tel: Slough 23971.

Guy's Hospital Medical School

#### GRADUATE RESEARCH ASSISTANT

required in the Department of Medicine to work for three years on the study of purine metabolism in uraemia. The post is suitable for a biochemist or enzymologist, preferably with a knowledge of chromatographic and electrophoretic techniques, automated analyses and the use of isotopes. Opportunity to register for higher degree.

Salary up to £2,022 per annum according experience, with Superannuation and Threshold Payments.

Apply in writing, with curriculum vitae, the Secretary, Guy's Hospital Medical chool, London Bridge, SEI 9RT, quoting ef. P.M. (2270)

#### UNIVERSITY OF MELBOURNE LECTURESHIP (LIMITED TENURE) IN THE DEPARTMENT OF ZOOLOGY

Applications are sought from Zoologists with interests and experience in the ecology of populations and communities.

Duties will include general undergraduate teaching in ecology at all levels, the major responsibility for a unit course in ecology in the second year, establishment of a research programme and the supervision of research students.

The appointee will be expected to take up duties as soon as possible after June 1, 1975.

Salary: \$A9,002 to \$12,352 per annum

Further information including details of application procedure and conditions of appointment is available from the Registrar, University of Melbourne, Parkville, Victoria 3052, Australia, of from the Association of Commonweakh Universities (Appts), 36 Gordon Square, London WCIH 0PF.

Applications close on January 31, 1975.

#### MEDICAL RESEARCH COUNCIL

TOXICOLOGY UNIT

#### TECHNICIAN/ JUNIOR TECHNICIAN

required in electron microscopy laboratory. Applicants for the Technician category must possess AIMLT or HNC or equivalent and preferably appropriate experience. Alternatively, applicants for the Junior Technician category may be considered and they should possess ONC or equivalent. Salary according to age, qualifications and experience. Apply in writing only to The Director, Toxicology Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey." (2267)

#### UNIVERSITÄT HOHENHEIM (LH)

A Chair of Plant Nutrition (AH 4) will be vacant as per 1.4.1976.

The new colleague will have to teach Plant Nutrition for students of General Agriculture, Agricultural Biology as we'll as Biology and to cooperate with two other professors in the department.

Concerning work he is expected to deal with physiological and ecological aspects of plant nutrition with special regard to yield and quality. Participation in the research programme "Ernährungsphysiologische Qualität landwirtschaftlicher Produkte" is desired.

Applications with the usual documents should arrive not later than 1.4.75 at the Dekan des Fachbereichs V der Universität Hohenheim (LH) 7 Stuttgart 70 (Hohenheim) Postfach 106

(2236)

#### UNIVERSITY OF SYDNEY CHAIR OF VETERINARY SURGERY

Applications are invited for the Chair of Veterinary Surgery which will become vacant on the resignation of Professor L. H. Larsen at the end of 1974.

The salary will be at the rate of \$A19,614 per

A statement of Conditions of Appointment and Information for Candidates may be obtained either from the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF, or from the Acting Registrar, University of Sydney, New South Wales 2006, Australia

ustralia.

Applications close on January 24, 1975.

(2238)

#### UNIVERSITY OF BENIN-NIGERIA

Applications are invited for the following posts in the DEPARTMENT OF CHEMISTRY:—

SENIOR LECTURER/

LECTURER IN THEORETICAL CHEMISTRY SENIOR LECTURER/ LECTURER IN INDUSTRIAL CHEMISTRY

SENIOR LECTURER/ LECTURER IN MATERIALS SCIENCE

(Polymer Chemistry, Metallurgy or Ceramics). Salary scales: Senior Lecturer N5,030 to N5,750. Lecturer N2,760 to N4,830 p.a. (£1 sterling=N1.44). The British Government may supplement salaries in range £750 to £1,150 p.a. (sterling) for married appointees or £250 to £50 p.a. (sterling) for single appointees (normally free of all tax) and provide children's education allowances and holidav visit passages. Current rates of supplementation are under review and results will be announced shortly. Revised rates will be effective from April 1, 1974. F.S.S.U. Various allowances. Application forms are available from the Registrar, University of Benin. PMB 1154, Ekwena Road, Benin City, Nigeria. Completed applications should be returned by air mail not later than January 17, 1975. Applicants esident in the \*UK should also send a copy to Inter-University Council, 90/91 Tottenham Court Road, London WIP ODT. Further particulars are available from either address. (2264) (Polymer Chemistry, Metallurgy or Ceramics).



### **Assistant** Librarian

with natural sciences qualification Ulverston, Cumbria.

An Assistant Librarian is required for our Ulverston factory, which manufactures antibiotics, enzymes and vitamins. The person appointed will join a small group giving a comprehensive library and information service to all departments of the factory.

The person appointed will be required to assist with the scanning of incoming literature, to undertake literature searches and compile bibliographies, answer enquiries, and assist with classifying additions to the library.

Candidates should have a professional qualification in librarianship or information science, with experience of working in a technical library, and should be graduates in one of the natural sciences.

The salary will be according to qualifications and experience. The company offers good conditions of service including bonus, pension and sick pay schemes.

Ulverston is a pleasant market town situated on the southern fringe of the Lake District National Park. Assistance with relocation expenses will be given where appropriate.



Please apply in writing quoting ref. U.691, to: The Personnel Officer (JE), Glaxo Laboratories Limited. North Lonsdale Road, Ulverston, Cumbria LA12 9DR. (2256)

### UNIVERSITY OF BIRMINGHAM THE DENTAL SCHOOL

A research team working on the biological evaluation of dental materials now needs a graduate in Biology or Zoology to undertake experimental implantation of materials under test and the assessment of their detailed effects upon dental and other tissues. The post, at the grade of RESEARCH ASSOCIATE or SENIOR RESEARCH ASSOCIATE is on a part-time basis, hours subject to negotiation, and would suit a married graduate with family responsibilities. No dental experience is required and the appointee will be given the necessary training on special dental aspects.

Salary pro rata on scale £1,758 to £3,285 (to £4,896 if candidate is exceptionally well qualified).

Further particulars from Assistant Registrar, Dental School, St. Chad's Queensway, Birmingham, B4 6NN, to whom applications including curriculum vitae and the names of three referees should be submitted within three weeks of the appearance of this advertisement. (2260)

#### UNIVERSITY OF OXFORD DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a postdoctoral research assistantship in metabolic biochemistry. The research will involve an investigation into the effects of ageing and certain drugs on some metabolic processes in the brain. The appointment is for two years at a starting date not later than May 1. 1975. The salary will be in the range £2,010 to £2,247 plus threshold payment and the person appointed will be eligible for membership of F.S.S.U. (or its equivalent).

Applications, with the names and addresses of two referees, should be sent to Dr E. A. Newsholme, Department of Biochemistry, South Parks Road, Oxford 0X1 3QU. The closing date for applications is February 14, 1975. (2271)

#### THE UNIVERSITY OF MANCHESTER DEPARTMENT OF MEDICAL BIOPHYSICS

Applications are invited for the post of Electron Microscopy Technician, Grade 5, in the Biophysics Laboratory of the Department. The person appointed will be responsible for the operation and day-to-day maintenance of three electron microscopes. He should have considerable experience and be acquainted with a wide range of specimen preparation techniques and photographic procedures and should be capable of assisting academic staff with research projects.

Applicants should have an H.N.C. or equivalent qualification. Salary scale £2,439 to £2,895 p.a. The post is superannuable.

Applications should be sent as soon as possible to Dr J. A. Chapman. Department of Medical Biophysics. Stopford Building, Manchester University, Oxford Road, Manchester M13 9PT.

(2285)

#### MEDICAL RESEARCH COUNCIL

**NEUROPSYCHIATRY UNIT** 

#### MORPHOLOGY ASSISTANT

required in Medical Research Council Neuropsychiatry Unit, with emphasis on electron microscopy. Experience in studies on the nervous system an advantage. Salary according to age and qualifications in range £1,860 to £2,835 plus £126 London Weighting.

Apply to Dr R. Balázs, Medical Research Council, Neuropsychiatry Unit, Woodmansterne Road, Carshalton, Surrey. (2266)

## Senior Bacteriologist from £4000

Ewans Biologicals Limited is one of the country's leading manufacturers of human and veterinary vaccines. Its modern premises are situated in Speke on the outskirts of Liverpool within commuter distance of Cheshire, The Wirral and Lancashire coast.

We now wish to appoint a Senior Bacteriologist to take charge of our pertussis vaccine programme when the present incumbent retires early in 1975.

We require someone who will probably have a post graduate qualification and considerable practical experience, with pertussis organisms and vaccines, so that we can maintain our eminence in this field.

Applicants should be able to justify a salary of at least £4,000. Salary will be negotiable above this according to qualifications and experience. Conditions of employment include a contributory pension scheme, the Glaxo Group profit based on bonus and assistance with relocation expenses where appropriate.

Please write, with brief career details, quoting reference U.54, to:



Dr M. C. Cook, Management Staff Adviser, Evans Medical Limited, Speke, Liverpool L24 9JD.

(2269)

SHEFFIELD AREA HEALTH AUTHORITY (TEACHING)
SHEFFIELD CENTRAL DISTRICT (TEACHING)

DEPARTMENT OF IMMUNOLOGY

### NON-MEDICAL SCIENTIFIC OFFICER

(Basic Grade)

required for a period of one year (in the first instance) for research into fetoprotein separation and quantitation.

Applicants should possess a 1st or 2nd Class Honours Degree in an appropriate subject. Experience in protein separation techniques and radioimmunoassay would be an advantage. For further details and arrangements to view the Laboratory, contact Dr A. M. Ward, Department of Immunology, Hallamshire Hospital, Sheffield. Telephone: (0742) 26484. Ext. 229.

Whitley Council Terms and Conditions of Service apply.

Salary Scale: £2,046 to £2,562 p.a. plus Threshold Agreement payments.

Applications in writing, stating age, qualifications, experience and the names and addresses of two referees to the District Administrator, Sheffield Area Health Authority (Teaching), Sheffield Central District (T), 10 Beech Hill Road, Sheffield S10 2RZ. (2276)

#### **PHARMACOLOGIST**

experienced, with veterinary or medical qualifications preferred, required for pharmacological research and toxicological studies of compounds of potential therapeutic importance. Also vacancies for New Graduates as trainees. Excellent opportunities for advancement in modern, well-equipped laboratory, Pension and Assurance Scheme. Application forms from: The Secretary, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London N1 2HB.

#### The Hannah Research Institute INFORMATION OFFICER/ LIBRARIAN

Applications are requested for the above post. The appointment will be within the Higher Scientific Officer grade (£2,461 to £3,371, plus threshold agreement). The post is pensionable. Applicants should possess a diploma or degree in science or agriculture and have a qualification in, or experience of, some aspect of information science. Applications, giving a full curriculum vitae and the names of two referees, should be sent to the Secretary, The Hannah Research Institute, Ayr. KA6 5HL, from whom further particulars may be obtained, by January 16, 1975. (2272)

#### FLATFORD MILL FIELD CENTRE

GRADUATES (females) wanted who would be interested in the post of Temporary Help (cooking) at Flatford Mill Field Centre whilst searching for permanent appointments. Could attend interviews and leave at short notice if necessary. Please write to The Warden, Flatford Mill Field Centre, East Bergholt, nr. Colchester, Essex or telephone (reverse charges) East Bergholt (020-629) 283. (2274)

#### MONASH UNIVERSITY Melbourne, Australia DEPARTMENT OF PHYSIOLOGY (INCLUDING PHARMACOLOGY) LECTURER/SENIOR TUTOR

The Department conducts courses for B.Sc. (Hons) and M.B., B.S. degrees and accepts graduate students for M.Sc. and Ph.D. degrees. Research interests include: brain ultra structure, neurophysiology, biophysics and pharmacology of central and peripheral synapses, muscle biophysics, circulatory physiology and pharmacology, renal and endocrine physiology. Applicants should be medically qualified or hold a Ph.D. in Physiology.

Salary scales (currently under review): Lecture.

Salary scales (currently under review): Lecturer (Medically qualified) \$A11,502 to \$A14,852. Lecturer \$A9,002 to \$A12,352 per annum, Senior Tutor \$A7,545 to \$A9,002 per annum, with superannuation based on an endowment assurance scheme, the employee and employer contributing 5% and 10% respectively.

respectively.

Benefits: Travelling expenses for appointee and family; removal allowance, repatriation after three years' appointment if desired: temporary housing for an initial period. For Lecturers only, study leave entitlement accumulates at the rate of one month's leave for each six months' service up to six years, with provision for financial assistance.

Further general information and details of appli-

Further general information and details of application procedures are available from the Academic Registrar, Monash University, Wellington Road, Clayton 3168, Victoria, Australia, or the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WCIH OPF, Enquiries about the Department to the Chairman, Professor R. Porter, in the University.

Closing date January 31, 1975.

The University reserves the right to make no appointment or to appoint by invitation.

#### FELLOWSHIPS AND STUDENTSHIPS

#### QUEEN ELIZABETH COLLEGE (University of London) DEPARTMENT OF CHEMISTRY DECCA RADAR POSTDOCTORAL RESEARCH FELLOWSHIP IN RADIOFREQUENCY SPECTROSCOPY

Applications are invited for a postdoctoral re-search fellowship in radiofrequency spectroscopy of the solid state, with particular reference to nuclear quadruple resonance.

Candidates should have a good research record in Physics, Chemistry or Chemical Physics and experience of and interest in instrumentation and computing would be an additional qualification.

This postdoctoral fellowship, tenable for two years, is supported by Decca Radar, Initial salary not less than £2,854 plus F.S.S.U.

Applications with curriculum vitae and the names of two referees should be sent to Professor J. A. S. Smith, Department of Chemistry. (N) Queen Elizabeth College, Campden Hill, London W8 7AH as soon as possible. (2244)

#### UNIVERSITY COLLEGE DUBLIN POSTDOCTORAL FELLOWSHIP IN PHYSICAL ORGANIC CHEMISTRY

Applications are invited for a Postdoctoral Fellowship to investigate intramolecular and bifunctional catalysis in R-eliminations with emphasis on analogies with biological reactions. The appointment, which is supported by the National Science Council of Ireland will commence on February 1, 1975 or as soon as possible thereafter. It will be renewable annually and in the normal way will extend to September 31, 1977.

Salary will commence at not less than £2,200

Applications should be sent to:
Dr R. A. More O'Ferrall
Department of Chemistry
University College Belfield Dublin 4, Ireland.

#### UNIVERSITY OF WATERLOO Waterloo, Ontaria, Canada AQUEOUS GEOCHEMISTRY-ISOTOPE GEOLOGY

Applications are invited for a postdoctoral fellowship in aqueous geochemistry-isotope geology. The successful applicant will have a strong background in low temperature geochemistry and possibly some experience in stable isotope and 14C research. The position is open immediately and the initial appointment is for one year but an extension to second year is possible. Salary according to N.R.C. regulations. Send résumé to:

Dr R. N. Farvolden, Chairman,
Department of Earth Sciences,
University of Waterloo,
Waterloo, Ontario, Canada,
N2L 3G1. (2249)

#### UNIVERSITY OF BIRMINGHAM DEPARTMENTS OF ANATOMY/ **PATHOLOGY** MEAD BEQUEST RESEARCH FELLOW

Applications are invited for the post of RE-SEARCH FELLOW to work under the joint supervision of Dr Martin Berry (Anatomy) and Professor W. Thomas Smith (Neuropathology) on an experimental study of regeneration in the central nervous system. This is a joint appointment for 3 years between the Department of Anatomy and Pathology and the extensive research facilities of both Departments will be available. Medical or non-medical graduates are invited to apply; previous experience of electronmicroscopy and/or amunofluorescent rechniques would be an advantage, though is not essential. The Fellowshin may be discussed with Professor W. T. Smith (021-472 1301 ext 3554). Department of Pathology, who will arrange for visits to the School.

Salary within the range of £2,118 to £2,931, according to age, qualifications and experience. Applications to Assistant Registrar, Faculty of Medicine, University of Birmingham, Birmingham B15 2TJ.

Closing date January 31, 1975. (2263)

Closing date January 31, 1975

(2263)

#### UNIVERSITY OF LIVERPOOL DEPARTMENT OF MEDICINE POSTGRADUATE RESEARCH STUDENTSHIPS

Applications are invited for Medical Research Council Studentships available in September, 1975.

Training is offered in a wide range of research activities principally in the fields of Immunology, Genetics, Drug Metabolism and Oncology, as related to medical problems.

Applications from students expecting to obtain II (1) Honours Science Degrees and wishing to take up Research Studentships for training in these subjects should be received not later than January 14, 1975 by the Registrar, The University. P.O. Box 147, Liverpool L69 3BX. Quote ref. RV/349/N. (2254)

#### UNIVERSITY OF WESTERN AUSTRALIA Perth

#### POSTDOCTORAL RESEARCH FELLOWSHIP IN MATHEMATICS

FELLOWSHIP IN MATHEMATICS

Applications are invited for a Postdoctoral Research Fellowship in the Department of Mathematics. The Fellowship is being financed by the Australian Research Grants Committee and the appointee will be required to work, in collaboration with Professor J. J. Mahony, in the general area of singular perturbation theory with some emphasis on establishing the validity of formal methods. It is expected that a research monograph will result. Opportunities will be available for continued personal research in the broad general area. Experience in appropriate methods of applied functional analysis and a good knowledge of the applications of singular perturbation theory are desirable. The appointee should be able to assume duty by mid-1975.

The initial appointment will be for one year

The initial appointment will be for one year with prospects of continuation for a further term. The current salary range is \$A9,002 to \$A12,352 p.a. An allowance towards appointment expenses will be available.

Applications in duplicate stating full personal Appecations in duplicate stating rull personal particulars, qualifications and experience should reach the Staffing Officer, University of Western Australia, Nedlands, Western Australia 6009, by January 18, 1975. Candidates should request three referees to write immediately to the Staffing Officer. (2283)

#### THE WELLCOME TRUST

TRAVELLING RESEARCH FELLOWSHIPS TO WESTERN AND EASTERN EUROPE 1975/76

Applications are invited from British re-search workers for the award of Wellcome-European Research Fellowships for 1975/76. These awards include the Trustees' special followships to Denmark, Germany, Hungary, Norway and Sweden.

Norway and Sweden.

The object of these fellowships is to encourage working visits to countries in Western and Eastern Europe by investigators in any branches of the natural and clinical sciences which have a bearing upon human or animal medicine. Candidates whose research is related to clinical problems will be given preference. These fellowships, which are normally for one year, are intended for graduates from the United Kingdom who already have some post-doctoral research experience. Stipends, according to age and experience, are within the range £2.120 to £4.165 per annum. A special cost of living allowance applicable to the country in which the candidate wishes to work is given where necessary. Travelling and some incidental expenses are provided in addition.

The candidate must make his own arrangements with the department in which he proposes to work, and written evidence must be submitted confirming his arrangements, Application forms may be obtained from the Assistant Director, The Wellcome Trust, No. 1 Park Square West, London, NW1 4LJ. Completed application forms must be returned by March 31, 1975. (2258)

#### UNIVERSITY OF WARWICK

Postdoctoral Research Fellowships in Biological Sciences/Molecular Sciences

Applications are invited for two postdoctoral fellowships to work with Professor D. C. Burke and Dr D. W. Hutchinson on the mechanism of interferon formation by polynucleotides. One post (financed by the M.R.C.) would be suitable for a biochemist with an interest in the use of affinity labelled compounds or in the structure and properties of eukaryotic membranes, and the other (financed by the C.R.C.) for a biochemist with an interest in virology or a virologist with a good background in biochemistry. Salary on the scale £2,118, £2,247, £2,412 (exceptionally £2,580) p.a. plus threshold payments. Further particulars and application forms may be obtained from the Academic Registrar. University of Warwick, Coventry SV4 7AL, quoting Ref. No. 19/2A/74. Closing date for receipt of applications is January 27,

#### AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for appointment to the following:

#### RESEARCH SCHOOL OF BIOLOGICAL SCIENCES PROFESSORIAL FELLOWSHIP DEPARTMENT OF GENETICS

The Department is headed by Professor W. F.R.S., and its research activities include the study of regulatory mechanisms in phage and bacteria, regulatory mechanisms and differentiation in lower eukaryotes, evolutionary studies on bacteria and vertebrates and developmental genetics of plant cell lines in tissue culture. The Department is well housed and equipped for these various fields of

It is now desired to make a senior appointment in the Department at the level of Professorial Fellow. The successful appointee, who should have demonstrated outstanding research ability, will have the opportunity of leading a small research group the Department, preferably within the field of plant cell genetics.

Closing date: February 7, 1975.

#### CENTRE FOR RESOURCE AND **ENVIRONMENTAL STUDIES** SENIOR FELLOW OR FELLOW

The primary role of the appointee will be, in collaboration with the staff and Advisory Committee of the Centre for Resource and Environmental Studies, to design a Master's course in Environmental Studies to be carried out primarily by course work, and to act as the course co-ordinator when the course is established. It is intended that students should be accepted in 1976. An opportunity will be avaisable for the successful applicant to carry out research related to current research projects of the research related to current research projects of the Centre.

There are no restrictions as to previous training and experience but the applicant should have research ability and some teaching experience. The applicant must be interested in environmental problems and in organising teaching at the graduate level.

Closing date: January 31, 1975.

Salaries (under review): The salary for a Pro-fessorial Fellow is \$A18,131 p.a. Salary on appoint-ment to the other posts will be in accordance with ment to the other posts will be in accordance with qualifications and experience within the ranges; Senior Fellow \$A14.724 to \$A16.921 p.a.; Fellow \$A10.771 to \$A14.704 p.a.; Current exchange rates are approximately \$A1:56p;\$US1.31.

Other Conditions: Tenure: Professorial Fellow to retiring age (65 years); Senior Fellow and Fellow for five years in the first instance with the possibility of extension to retiring age.

Reasonable travel expenses are paid and assistance with housing is provided for an appointee from outside Canberra, Superannuation is on the F.S.S.U. pattern with supplementary benefits.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should write to the Association of Commonwealth Universities (Appis), 36 Gordon Square, London WCIH 0PF for further particulars before applying.

#### UNIVERSITY OF BIRMINGHAM DEPARTMENT OF BIOCHEMISTRY

RESEARCH ASSOCIATE OR RESEARCH FELLOW

Applications are invited for the above post for research on the occurrence, effects and control of lipid peroxidation processes in mitochondrial membranes from normal and cancer cells. A preferred and experience in the biochemistry of lipids and mitochondria an advantage.

Salary scales: Research Associate £1,758 to £2,412; Research Fellow £2,118 to £2,412 both plus F.S.S.U. and threshold payments.

Applications, including names of two referees by January 31, 1975 to Assistant Registrar (RS). University of Birmingham, P.O. Box 363, Birmingham B15 2TT. Further particulars are available from Dr A. A. Horton, Department of Biochemistry, Please quote ref. BN. (2262)

#### UNIVERSITY OF BIRMINGHAM DEPARTMENT OF EXPERIMENTAL PATHOLOGY

A Research Fellow is required to join a research team under M.R.C. sponsorship presently working on carcinoembryonic antigen and cell mediated tumour immunity on patients with cancer and other diseases. Applications are invited from graduates with research qualifications and experience in related fields, Salary: £2,118 to £2,412 plus F.S.S.U.

Enquiries and applications should be made to Dr P. W. Dykes, Department of Experimental Pathology, University of Birmingham, Birmingham B15 2TJ. (AK2278)

#### THE UNIVERSITY OF ASTON IN BIRMINGHAM DEPARTMENT OF PHARMACY RESEARCH IN PHARMACEUTICAL **SCIENCES**

The Department has recently attracted substantial research funds from Industry, the Research Councils and other institutions and has particularly close links with Pharmaceutical Industry and the West Midlands Regional Health Authority. As part of a general expansion of research activities in the Department, the following vacancies are available:

#### POSTGRADUATE STUDENTSHIPS

These awards (based on S.R.C. provisions) are available in Medicinal Chemistry, Microbiology, Pharmaceutics and Pharmacology and successful applicants will register for a Higher Degree, Recent graduates in Pharmacy or any appropriate discipline, and students who anticipate graduating in 1975 are invited to visit the Department or write to the Postgraduate Tutor, Dr M. F. G. Stevens, for further information.

#### RESEARCH FELLOWSHIP IN PHARMACEUTICAL BIOLOGY (Ref. No. 009)

The successful candidate will work on resistance of Gram-negative organisms and preference will be given to candidates with experience in the biochemistry of bacterial envelopes.

The appointment will be for a period of two years and the commencing salary will be within the range £2,118 to £2,412 per annum.

Intending applicants may obtain information on the nature of the work from Professor M. R. W. Brown, Department of Pharmacy, Application forms and other details may be obtained from the Staff Officer (quoting Ref. No. 009/6), the University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET to whom applications should Birmingham B4 7ET to wnom appearance of be returned within 14 days of the appearance of (2279)

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#### THE NATIONAL KIDNEY RESEARCH **FUND**

invites applications for grants for renal research. at units within the United Kingdom. Application forms are available from The Secretary, 184B Station Road, Harrow, Middlesex HA! 2RH (01-836 4469). (2268)

#### AUSTRALIAN NATIONAL UNIVERSITY

## SCHOLARSHIPS FOR Ph.D. DEGREE COURSES

Persons, who hold, or expect to hold, a Bachelor degree with at least upper second-class honours or equivalent from a recognised university and who have a capacity for research, are invited to apply for Australian National University Ph.D. Scholarships. These are tenable in the Institute of Advanced Studies, in the School of General Studies or in the Computer Centre and are offered in the fields of study listed below:

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Botany Chemistry

Developmental Biology Environmental Biology Experimental Pathology

Forestry Genetics

Geomorphology

Human Biology (Human Genetics, Urban Biology)

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Microanalytical Chemistry

Microbiology
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Neurobiology
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Applications: Application forms and further particulars are available from the Academic Registrar, The Australian National University, P.O. Box 4, Canberra ACT 2600, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF.

There is no set closing date. Applicants from outside Australia are advised to apply, stating field of interest, at least six months before they would expect to be able to take up a Scholarship, if offered.

Completed applications from overseas should be sent direct to the University and not to the nearest Australian Embassy, High Commission or Consulate. Successful applicants will be notified of any requirements which they will have to satisfy for entry into Australia as private students. (2239)

#### LECTURES AND COURSES

### UNIVERSITY OF ABERDEEN DEPARTMENT OF MEDICAL PHYSICS

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Further particulars from: Professor J. R. Mallard, Ph.D. D.Sc., F.Inst.P., C.Eng., M.I.E.E., F.R.S.E., Department of Medical Physics, The University, Aberdeen, (2273)

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Applications should reach J. Tooze, Executive Secretary, European Molecular Biology Organisation, 6900 Heidelberg, Postfach 1022.40, West Germany, before January 31, 1975. They should include a curriculum vitae, and an indication of the applicant's scientific background and current interests. Applicants will be informed whether or not they can be accepted during March 1975. A registration fee of 40 DM will be charged. Accommodation in Hirschhorn (15 DM to 30 DM per night) will be arranged by the Symposium Secretariat but participants will be responsible for their living and travel expenses. A small number of fellowships are available which will be awarded by the Organising Committee to young participants unable to obtain other sources of support. Persons wishing to compete for these few fellowships should so indicate in their letter of application.

The Organising Committee:

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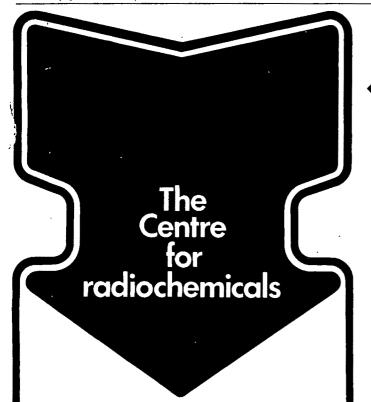
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